• Volume 8D, Number 3–4, April 1960

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section D BOTANY

Bull. Res. Counc. of Israel. D. Bot.

Continuing the activities of the
Palestine Journal of Botany,
Jerusalem and Rehovot Series

Page

The Weizmann Science Press of Israel • Jerusalem • ירושלים • מוסד ויצמן לפרסומים במדעי הטבע ובטכנולוגיה בישראל

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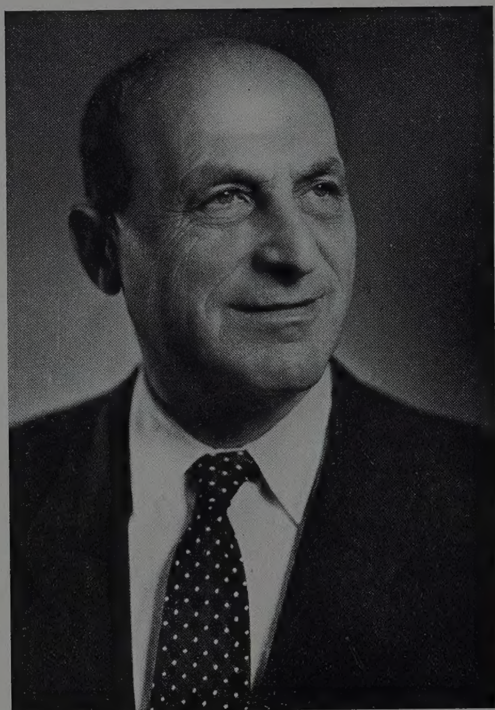
On the occassion of
the 60th birthday of
PROFESSOR HILLEL REINHARD (HEINZ) OPPENHEIMER



PROFESSOR HILLEL REINHARD (HEINZ) OPPENHEIMER

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At the session held on January 26, 1922, in the Section of Mathematics and Natural Sciences of the Vienna Academy of Science, Professor Hans Molisch, the famous plant physiologist of Vienna University, presented a short communication by his pupil, the 22-year-old student Heinz Oppenheimer. It demonstrated the presence of germination inhibitors in the pulp of fleshy fruits such as tomatoes and cucumbers. The doctorate thesis on the same subject which was published later, was indicative of the main features of the scientific life work of the present Professor Hillel Oppenheimer. It showed a basic approach to problems, a critical evaluation of methods and a sense for the practical application of science.

These qualities Oppenheimer certainly inherited from his great father, Professor Franz Oppenheimer (1864—1943), the renowned sociologist of the Frankfurt University, who endeavoured to realize his ideas of social justice and agrarian reform by the establishment of cooperative settlements. To no lesser degree, Oppenheimer was influenced by his teacher Molisch who devoted his life work to pure as well as to applied research.

His inclination to practical aspects of botanical problems expressed itself also in his love for gardening. Discharged from service in the German army where he served from 1917 to 1919 at the eastern front, he studied botany at German universities under Haberlandt, v. Guttenberg, Mobius and Oltmanns. Through all his studies, he worked as an apprentice or assistant gardener in various gardening establishments, thus acquainting himself thoroughly with horticultural practice.

To these factors which moulded his scientific character, one may add his inclination gradually maturing in him after the war, to help develop a prosperous Jewish agriculture in Palestine. Agricultural progress in a dry country means helping plants in their struggle against drought. Thus, water relations and drought resistance of plants became the keynote of his scientific life work.

In 1924 he entered the famous Spaeth nurseries near Berlin as gardener. Soon after, he was appointed head of the firm's plant protection service. Here, he developed methods for the control of the so far little-studied nursery diseases and pests. He performed successful experiments in controlling crown-gall of fruit trees.

Declining a flattering offer as Scientific Adviser in Horticulture to the Spaeth Nurseries, Oppenheimer left Germany and set out for Palestine in 1925. The summer of 1926 was spent working in the nursery of Dr. S. Soskin's Smallholders' Colonisation Company in the Kabbara swamps near Caesarea.

In the following autumn, Oppenheimer accepted an invitation from the Aaronsohn family in Zichron Jaakov to care for the large plant collection of the late agronomist Aaron Aaronsohn, the famous discoverer of wild wheat. Oppenheimer engaged in this work with great enthusiasm as it offered him the opportunity of acquainting himself with the flora of Palestine and its geobotanical problems.

Only in 1931, three years after The Hebrew University of Jerusalem had opened a Department of Botany, Oppenheimer had the opportunity to get nearer to his life goal—to study plants in their relation to water. Here, in the inspiring atmosphere of Mt. Scopus, in the summer months of 1931, he laid the foundations to his critical studies on the water balance of plants and undertook his first investigations into the water physiology of forest trees of the Mediterranean area.

In 1933, Oppenheimer was called by the Agricultural Research Station, Rehovot, to join its staff as a physiologist and later as a citriculturist. Here he succeeded to apply the experimental methods of water ecology to the solution of irrigation problems of the citrus tree.

In 1942, Oppenheimer was appointed Head of the Division of Citriculture at the Institute of Agriculture of the Hebrew University at Rehovot, now the Faculty of Agriculture. Associate Professor since 1949, he became James de Rothschild Professor of Horticulture in 1952 and Professor

of Plant Physiology in 1957. In the faculty, he initiated teaching and research in citriculture, floriculture, silviculture and dendrology. His latest appointment allows him to return to his original studies in the theory of water balance and drought resistance.

For a clear evaluation of Oppenheimer's research activities, it seems appropriate to treat the subject under seven headings: (1) methodological studies, (2) water balance, (3) Near East botany, (4) history of plant science, (5) ornamentals, (6) forestry, (7) citriculture.

(1) *Methodological studies*. This branch of Oppenheimer's studies best demonstrates his high scientific capabilities, of critical thoroughness and constructive ingenuity. Very few contemporaneous water physiologists have contributed so much to the clarification of notions and methods used in studies of water balance.

At the beginning of Oppenheimer's scientific career, most plant physiologists were deeply impressed by Fitting's discovery in the Algerian desert (1911) that wilting of plants can be prevented or reversed by extremely high osmotic pressures of their cell fluids. These results confirmed by Stocker (1928) in the Egyptian desert were obtained with de Vries' plasmolytical method, but seemed to differ markedly from results obtained by cryoscopy of expressed cell sap practiced by English and American botanists. Oppenheimer undertook simultaneous measurements with both methods which rendered good agreement in most cases, but discrepancies in others. These seemed to occur in plants with a viscous protoplasm which required very long times for plasmolysis. For these, the cryoscopic method would be preferable and Fitting's figures too high.

Also the figures obtained with Ursprung and Blum's much admired methods for the measurement of suction tension in cells and organs aroused his suspicion. They appeared unreasonably high if compared with osmotic pressures, as also found by Walter. Oppenheimer based his criticism on a study of a neglected determinant of osmotic cell qualities: the extensibility of the cell wall. In contrast to Ursprung's assumptions, it was very small or non-existant in fully developed cells and did not obey Hooke's law of a direct proportionality between the extending force and the increment in volume or length. Ursprung's and Blum's measurements were thus shown to need reexamination, since in many cases they misinterpreted experimental results.

A critical examination of Ursprung's "strip method", much used by ecologists, proved not less disappointing with the studied leaves. Better results were obtained with prisms of storage roots and tubers first introduced by Oppenheimer during his stay in Pringsheim's laboratory in Prague and now generally used in students' laboratories for the measurement of the "diffusion pressure deficit". During his stay in Prague, Oppenheimer also coined the term "osmotic potential" of cell fluids and cells, which is now in general use for pressure.

Very important work has been done by Oppenheimer and his pupils, Nadel and Shmueli, to elucidate the value of different methods for the measurement of stomatal aperture in leaves—a very useful indication of irrigation requirements. Lloyd's alcohol fixation method failed completely with citrus leaves for which it had been widely used. Replacing it by Molisch's injection method, good results were obtained. Further studies led to the conviction that each plant requires the adaptation of the method, suiting it by preliminary studies.

As a direct measure of water deficiency in plant organs, the actual "water saturation deficit" which Stocker determines by saturation in a closed atmosphere attracted his attention. The method which rendered no uniform results with sclerophyllous leaves, was checked against Weatherley's method of punched leaf disks floating on water by Oppenheimer's pupil Nitzan. Each method was found to have its drawbacks which must not be overlooked.

Oppenheimer's most important contribution to the methodology of water relations was, however, the concept of a "sublethal" water saturation deficit indicating the tolerable limit of dehydration. He is now engaged in improving the method of its determination by cell physiological studies.

Hardly less important were Oppenheimer's studies on water absorption from the soil. He challenged the accepted opinion of Briggs and Shantz, of Hendrickson and Veihmeyer that soil moisture is equally available to plants between the permanent wilting percentage and the field capacity. Using various physiological methods, like measurement of fruit growth and stomatal aperture, he proved the fallacy of their view. His conclusion that, as maintained by classical plant physiology, water becomes gradually less available when the soil dries, is now accepted by most physiological authorities.

(2) *Water balance.* Oppenheimer's first plant ecological contribution was his article "Zur hochsommerlichen Wasserbilanz mediterraner Geholze" (1932). It meant a great step forward in the study of xerothermic vegetation, since the author used well checked old and well invented new methods in a general "clinical" examination of the plant's present status. In this study, which is considered classical, he showed that some evergreens can reduce transpiration practically to zero, by stomatal regulation. Confirming his findings in further studies, he coined new terms for transpirational behaviour.

(3) *Near East Botany.* The fruit of Oppenheimer's work in Aaronsohn's collection which lasted from 1926 to 1938 and was resumed in 1953, were two volumes of determinations and plant descriptions, one of the flora of Transjordan and the other on Cisjordania, the latter together with M. Evenari. He further published a plantgeographical sketch of Transjordania, and, with Evenari, a contribution to the flora of the Bosporus. In continuation of Aaronson's botanical achievements Oppenheimer treated the problem of the origin of cultivated wheat from wild emmer in the light of recent genetic investigations. He also settled the long debated problem of the origin of the Egyptian Clover which he found growing in Israel.

Oppenheimer has continued also with botanical studies on taxonomic or plant geographical subjects. Thus he undertook a floristic and sociological study on the light and heavy soil vegetation in the coastal region near Jaffa which was doomed to destruction by drainage. He studied the Hula swamp vegetation, and took interest in the flora of the desert near the Dead Sea. Not less interesting were his ecological studies on the influence of light and frost on cultivated and spontaneous plants, and of roots growing in unfissured rocks.

(4) *History of botany.* Oppenheimer revealed a special gift in depicting the scientific life history of great botanists. His biographies of de Vries, his teacher Molisch and Vochting (unpublished) are outstanding, embracing important phases in the history of plant physiology. His accounts of the work of Eig and Aaronsohn (the latter in Hebrew and not included in the bibliography) are also vivid and most interesting.

(5) *Forestry.* Oppenheimer's first contributions to forest ecology date back to his activities on Mt. Scopus (1931-33) where he ran a forest nursery and a tunnel for observations on root growth. His activities assumed a more pronounced silvicultural character during his work at the Agricultural Research Station. His main attention was devoted to the xerothermic Aleppo Pine. He studied its physiology of germination, root and shoot growth, cambium activity, soil adaptation and other aspects. During many years, he tried to elaborate a mehod of afforestation for the local oak species. Water use and root structure of the East mediterranean forest vegetation were investigated, and growth rates, natural reproduction and ecological adaptation of exotics were studied.

(6) *Ornamentals.* Floriculture and landscape gardening have made great progress in Israel since the establishment of the State. Oppenheimer encouraged his pupils Monselise and Halevy to undertake floricultural research and teaching at the agricultural faculty while he himself cooperated in the introduction of new species and rose varieties. His experiments on the cultivation of tropical orchids contributed basic data of great floricultural interest.

(7) *Citriculture.* Oppenheimer's citricultural studies present, so far, the most complete and extensive chapter of his life-work. They may be considered as outstanding in the literature on citrus growing. From 1942 to 1952 he devoted nearly all of his time to citrus problems. As the highlights of these studies embracing all the phases of citrus cultivation we consider two treatises—his bioclimatic study of orange leaf transpiration (with Mendel) and his study on irrigation of citrus trees (with Elze). They helped to create a firm biological foundation in the theory and practice of irrigation of subtropical plantation crops. Others like Shmueli working with bananas and Patt with citrus solved the irrigation problems of these crops, developing Oppenheimer's methods further.

The same must be said in respect of citrus nutrition and fertilisation. He investigated lime and potash requirements and introduced leaf analysis as a practical tool into citricultural practice. His pupil, the late Dr. Lotte Heymann-Herschberg developed this line of studies further, extending it to more citrus varieties and to the micronutrients.

Of great practical importance for Israel's citriculture were Oppenheimer's successful experimental studies to save citrus plantations badly neglected during World War II by appropriate pruning. He also succeeded in improving the fruitfulness of the Clementine variety by interplanting it with mandarins, thus inducing effective pollen fertilization.

Oppenheimer's original citricultural research was incorporated by him in a textbook on citrus growing written for local growers which, if translated, would occupy an honourable place among the existing textbooks on citrus growing.

Not less outstanding than his scientific production must be considered Oppenheimer's achievements as a teacher and educator. Many pupils trained by him first at the Department of Botany of The Hebrew University where he lectured plant anatomy and physiology since 1932, and later on at the Faculty of Agriculture, continue in the various fields of his scientific and pedagogical activities or work as agricultural teachers or instructors, bringing up a young generation of learned farmers, or guiding agricultural settlements and raising their professional level. The contributions of his pupils to science are too numerous even to be enumerated here. He took great pains to revise their texts thoroughly and to see them all published.

Professor Oppenheimer may look back on his scientific life-work with great satisfaction. The dream of his young days to synthesize the activities of a plant physiologist and a horticulturist has become true. But he has achieved also a synthesis of plant physiological and plant geographical knowledge, as his recent review for Unesco demonstrates and has given his share to the reconstruction of the Land of his Fathers. Recently, he has earned a highest token of recognition from the reborn Jewish nation—the Israel Prize in Agriculture. He is also held in high esteem all over the botanical world.

It is again pure physiological investigation of plant cells and the foundations of their drought resistance which capture his imagination and time—searching for new methods and discoveries. We wish splendid success and good health to him and to his flourishing family!

I. Reichert

LIST OF PUBLICATIONS OF PROF. HILLEL (HEINZ) R. OPPENHEIMER

1. Keimungshemmende Substanzen in der Frucht von *Solanum Lycopersicum* und in anderen Pflanzen. *Sitzb. Akad. Wiss. Wien, Abt. I,* **131**: 59–65, 1922.
2. Das Unterbleiben der Keimung in den Behältern der Mutterpflanze. *Sitzb. Akad. Wiss. Wien, Abt. I,* **131**: 279–312, 1923.
3. Verhütung und Heilung krebsartiger Pflanzengeschwülste. *Angew. Bot.* **8**: 8–29, 1926.
4. Die Therapie der Baumschulkrankheiten. *Angew. Bot.* **80**: 137–146, 1926.
5. Physiologische Probleme bei der Citrus-Anzucht. *Angew. Bot.* **10**: 103–109, 1928.
6. Ueber die Flora des Ostjordanlandes. *Ber. dtsch. bot. Ges.* **48**: 1–3, 1930.
7. Dehnbarkeit und Turgordehnung der Zellmembran. *Ber. dtsch. bot. Ges.* **48**: 192–206, 1930.
8. Kritische Betrachtungen zu den Saugkraftmessungen von Ursprung und Blum. *Ber. dtsch. bot. Ges.* **48**: (130)–(140), 1930.
9. Reliquiae Aaronsohnianae I. Florula transiordanica. *Bull. Soc. bot. Genève* **22**: 124–292, 1930 (published 1931). With the diaries of Aaronsohn. Published also as a book in a Hebrew edition, 1935.
10. Esquisse de géographie botanique de la Transjordanie. *Bull. Soc. bot. Genève* **22**: 410–438, 1930 (published 1931).
11. Ueber Zuverlässigkeit und Anwendungsgrenzen der üblichsten Methoden zur Bestimmung der osmotischen Konzentration pflanzlicher Zellsäfte. *Planta, Arch. f. wiss. Bot.* **16**: 467–517, 1932.
12. Zur Kenntnis der hochsommerlichen Wasserbilanz mediterraner Gehölze. *Ber. dtsch. bot. Ges.* **50a**: 185–245, 1932.
13. Untersuchungen zur Kritik der Saugkraftmessungen. *Planta* **18**: 525–549, 1932.
14. Studien zur Keimung und ersten Entwicklung der Aleppokiefer und Kermeseiche. *Gartenbauwissenschaft*: 308–364, 1933.
15. Some experiments on water relations of citrus trees (in collab. with K. Mendel). *Hadar* **7**: 35, 59, 150, 1934.
16. Problems of citrus nutrition. I. On lime nutrition of citrus trees. *Hadar* **7**: 268–271, 1935; **8**: 11–13, 1935.
17. The present state of botanical, horticultural and sylvicultural research in Palestine. *Palest. J. Bot. hortic. Sci.* **1** (1): 8–21, 1935.
18. Critical remarks on the value of Lloyd's alcohol fixation method for measuring stomatal aperture. *Palest. J. Bot. hortic. Sci.* **1** (1): 43–47, 1935.
19. Hugo de Vries als Pflanzenphysiologe. *Palest. J. Bot. hortic. Sci.* **1** (1): 51–69, 1935.
20. A citrus root stock trial on light soil. *Hadar* **9**: 35–40, 1936.
21. Etudes sur le développment des racines de quelques plantes méditerranéennes. *Silva mediterranea* **10**: 142–162, 1936.
22. Remarks on two recent critical contributions concerning methods used in plant physiology. *Palest. J. Bot. hortic. Sci.* **1** (2): 84–93, 1936.
23. Un nouveau *Delphinium* palestinien de l'Herbier Aaronsohn. *Bull. Soc. bot. Genève* **26**, 6 pp., 1936.
24. Injurious salts and the ash composition of fruit trees. *Hadar* **10**: 91–94, 125–128, 133, 1937.
25. How to produce summer lemons in Palestine? *Hadar* **11**: 231–237, 1938.
26. Reliquiae Aaronsohnianae III. Une contribution à la connaissance de la flore du Bosphore (in collab. with M. Evenari). *Palest. J. Bot. Rehovot* **2**: 17–33, 1938.
27. An account of the vegetation of the Huleh swamps. *Palest. J. Bot. Rehovot* **2**: 34–39, 1938.
28. In memory of Hans Molisch. *Palest. J. Bot. Rehovot* **2**: 98–112, 1938.
29. Problems of citrus nutrition II. Studies on the potash requirement of sweet lime (in collab. with K. Mendel). *Hadar* **11**: 177–181, 244–248, 1938.
30. In memoriam Alexander Eig. *Palest. J. Bot. Rehovot* **2**: 145–152, 1939.
31. Orange leaf transpiration under orchard conditions. Part I. Soil moisture content high. A bioclimatic study (In collab. with K. Mendel). *Palest. J. Bot. Rehovot* **2**: 171–250, 1939; also *J.A.P. agric. Res. Sta. Bull.* **25**.
32. Reliquiae Aaronsohnianae II. Florula cisiordanica (in collab. with M. Evenari). *Bull. Soc. bot. Genève* **31**, 432 pp., 12 pls., 1940 (published 1941). With the diaries of Aaronsohn. Published also as a book in a Hebrew edition.
33. Etudes sur le problème de la reconstitution de chênaies en Palestine. *Palest. J. Bot. Rehovot* **3**: 105–143, 1940.
34. A contribution to the desert flora South and South-West of the Dead Sea. *Palest. J. Bot. Rehovot* **3**: 144–153, 1940.
35. The stock problem of the Shamouti orange. *Hadar* **13**: 245–248, 1940.
36. How to produce summer lemons in Palestine. II. *Hadar* **13**: 169–170, 1940.
37. Root cushions, root stalagmites and similar structures. *Palest. J. Bot. Rehovot* **4**: 11–19, 1941.
38. Irrigation of citrus trees according to physiological indicators (in collab. with D.L. Elze). *Palest. J. Bot. Rehovot* **4**: 20–46, 1941; also *J.A.P. agric. Res. Sta. Bull.* **31**.
39. Rejuvenation of citrus groves by cutting back the main limbs of the trees (in collab. with H. Weizmann). *Hassadeh* **219**: 286–287, 1941.

40. The problem of the neglected citrus grove and its management. *Hassadeh* **23**: 129–131, 1943.
41. Cambial wood production in stems of *Pinus halepensis*. *Palest.J.Bot.Rehovot* **5**: 22–51, 1945.
42. Leaf analyses of Shamouti oranges. *Palest.J.Bot.Rehovot* **5**: 86–95, 1945.
43. Some cases of uncommonly rapid growth in Palestine. *Palest.J.Bot.Rehovot* **5**: 112–123, 1945.
44. Twenty years of citricultural research at the J. A. P. agric.Res.Sta.Rehovot. A critical survey, 35 pp., 1946 (unpublished).
45. Natural reproduction of exotic plants in Palestine. *Palest.J.Bot.Rehovot* **6**: 14–19, 1947.
46. Studies on the water balance of unirrigated woody plants. *Palest.J.Bot.Rehovot* **6**: 63–77, 1947.
47. Experiments with unfruitful Clementine mandarines in Palestine. *J.A.P.agric.Res.Sta.Rehovot, Bull.* **48**, 63 pp., 1948.
48. Sand, swamp and weed vegetation at the estuary of the Rubin River (Palestine). *Vegetatio* **1**: 155–174, 1948–49.
49. Frost effects on vegetation in 1948/49. *Palest.J.Bot.Rehovot* **7**: 36–40, 1949.
50. La détermination de l'ouverture stomatique chez la tomate. *Palest. J. Bot.Rehovot* **7**: 63–68, 1949.
51. The water turn-over of the Valonea oak. *Palest.J.Bot.Rehovot* **7**: 177–179, 1949. Published also in *Haya'ar* **2**: 30–32, 1950 in Hebrew.
52. Studies on the reestablishment of citrus groves during the Second World War. *J.A.P.agric.Res. Sta.Rehovot, Bull.* **54**, 52 pp., 1950 (in Hebrew).
53. Summary of the investigations on forest tree life in Israel. In '*Netiv Hahagshama*', a volume in honour of Joseph Weitz: 53–66, Keren Kayemeth Leisrael, Jerusalem, 1950.
54. Plant reactions as indicators of irrigation requirement. *Proc. 7th intern. bot. Congress, Stockholm* 1950: 172–173, 1950.
55. Summer drought and water balance in the Near East. *Proc. 7th intern. bot. Congress, Stockholm* 1950: 235–236, 1950.
56. The physiology of irrigated plants. In '*Hamaim Vehakarka*' (The Water and the Soil): 141–155, Tel-Aviv, 1951 (in Hebrew).
57. Regeneration of maquis after fire. *Haya'ar* **3**: 9–12, 1951 (in Hebrew).
58. Summer drought and water balance of plants growing in the Near East. *J. Ecology* **39**: 356–362, 1951.
59. Light evading plants in the hills and plains of Israel. *Palest.J.Bot.Rehovot* **8**: 20–26, 1951.
60. Physiological behaviour of maize under irrigation. *Palest.J.Bot.Rehovot* **8**: 32–44, 1951.
61. Geobotanical research in Palestine, 1938–1950. *Vegetatio* **3**: 301–320, 1952.
62. Water requirement and irrigation of citrus groves. *Rep. of the 12th intern. hortic. Congress, London* 1952: 1267–1274, 1952.
63. An experimental study on ecological relationships and water expenses of Mediterranean forest vegetation. *Palest.J.Bot.Rehovot* **8**: 103–124, 1953.
64. The ecological character of *Rumex Rothschildianus* Aaronsohn. *Palest.J.Bot.Rehovot* **8**: 158–162, 1953.
65. Effect of nitrogen fertilizing on soil, growth, leaf composition and yield in the Shamouti orange groves (in collab. with L. Heymann-Herschberg). *Ktavim* **5**: 5–17, 1954.
66. Pénétration active des racines de buissons méditerranéens dans les roches calcaires. *8ème Congrès intern. de Botanique, Paris* 1954, *Sect.* **27**: 173, 1954.
67. Critique expérimentale de deux methodes employées en vue d'établir le déficit de saturation hydrique (DSH) des feuilles (in collab. with J. Nitzan). *8ème Congrès intern. de Botanique, Paris* 1954, *Sect.* **11–12**: 218–220, 1954.
68. *Water Metabolism of the Plant* (in collab. with K. Mendel). Tel-Aviv, 43 pp., 1955.
69. Pénétration active des racines de buissons méditerranéens dans les roches calcaires. *Bull.Res. Counc.of Israel* **5D**: 219–222, 2 pls., 1956.
70. The influence of the soil on the development and the mineral composition of the Aleppo pine. Analyse des plantes et problèmes de fumures minérales I.R.H.O. Paris 1956: 272–280, 1956.
71. *Citriculture*, Hassadeh Libr. Tel-Aviv, 295 pp., 1957 (in Hebrew).
72. Wild emmer - its discovery by A. Aaronsohn fifty years ago, and its importance for the origin of cultivated wheats. *Svensk bot. Tidskr.* **51**: 545–551, 1957.
73. Cinquante ans après la découverte du blé sauvage par A. Aaronsohn: L'importance de la découverte pour le problème de l'origine des blés cultivés. *Bull.Soc.bot.France* **104**: 293–297, 1957.
74. Further observations on roots penetrating into rocks and their structure. *Bull. Res. Counc. of Israel* **6D**: 18–32, 1957.
75. Plant adaptation to drought. *Hammadah* **2**: 35–42, 1957.
76. Adaptation des agrumes â la sècheresse. *Livre du IVème Congrès de l'agrumiculture méditerranéenne*, Mai 1956, Tel-Aviv: 232–238, 1958.
77. The origin of the Egyptian clover - with critical revision of some nearly related species. *Bull. Res. Counc. of Israel* **7D**: 202–222, 1959.
78. Adaption to dryness — xerophytism. Arid Zone Research, UNESCO, Paris, 1960 (in press).
79. Observations botaniques dans des orangeries abandonnées. (Sent for publication).

INVESTIGATIONS ON WATER RELATIONS OF SOME INDIAN CROP PLANTS—A REVIEW

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ABSTRACT

On account of the seasonal nature and relatively short duration of rainfall in India, information on the water relations of crop plants is important for the proper utilization of limited water supply. The results of comparatively recent investigations on the water relations and drought resistance of some important crop plants like rice, jowar, sugarcane, wheat and cotton have been briefly reviewed and a few suggestions regarding characters to be studied in respect to assessment of drought resistance of varieties have been offered.

INTRODUCTION

The striking features about rainfall in India are its seasonal nature and relatively short duration. By far the largest portion of the annual rainfall is received during the south-west monsoon. Small amounts of rainfall are also received during winter and spring in a few parts of the country but their general effectiveness is uncertain. We might regard less than 75 cm as low rainfall, considering the general high level of mean annual temperature, and this, apart from its large annual variability, is characterized by a much larger variability in its monthly distribution. It has been estimated that nearly one-third of the total cultivable area is situated in the low rainfall zone and that even with full provision of irrigation facilities from all sources it may not be possible ultimately to bring more than 60% of the total cultivable land under irrigation. Hence application of techniques of dry farming assume importance and in this context information on water relations of crop plants would be of considerable significance. It would, therefore, seem timely to assess briefly the present status of our information on this topic.

Among the earliest investigations, those of Leather (1910, 1911) on water requirements of crop plants, of Dastur (1924) on the effect of water content of leaf on photosynthesis, of Inamdar and Dabral (1930) on water balance and of Singh *et al.* (1935, 1937, 1938, 1939) on water requirements may be mentioned. It is proposed to review here comparatively more recent work on some important crop plants.

The methods adopted to estimate evaporation and potential evapo-transpiration over the Indian sub-continent and the preliminary results obtained have been discussed recently by Ramdas (1957).

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RICE

Only about 20% of the rice area has irrigation facilities to supplement the water received from rainfall and the main cause of the general low level of yield appears to be erratic rainfall.

Water requirement: The transpiration ratio has been found to be approximately 540 and to vary directly with the growth period of variety. The estimate of quantities of water required for different growth stages is: 1 acre-inch for seed-bed, 22 acre-inches for transplanting and 37 acre-inches for maturing (Ghose *et al.* 1956).

Flood resistance: Large tracts of rice-land, particularly in Assam, Bengal, western India, Orissa, Madras and Andhra, are subject to floods during the rainy season and the crop is submerged for varying lengths of time. Some varieties grow in height with the rise in water level and thus keep above the surface while others appear to withstand submergence. Physiological investigations were carried out along two lines. Sen (1937) and Ghosh (1949, 1954, 1959) raised plants in pots (soil culture) and submerged the latter in varying depths of water in cement tanks. It was observed that: (i) water above soil level suppressed tillering and yield; (ii) the sugar content of leaves increased with increase in depth of submergence due probably to its decreased utilization in tillering which was suppressed; (iii) the uptake of nitrogen by the plant also decreased when the water level was 3 to 6 inches above the soil level. Parija and Pillay (1944) attempted to induce flood resistance by pre-sowing treatments such as (a) subjecting germinated seeds to temperature of approximately 5°C for 6 days, (b) exposure of germinated seeds to a continuous current of O_2-free air for 3 days and (c) a and b combined. The seedlings (in pots) were then submerged in water for 2 to 3 weeks and subsequently their survival was followed under normal conditions. The percentage survival of the seedlings treated by any of the three methods was found to be greater than that of the untreated ones.

Drought resistance: Parija and Pillay (1945) found that plants of summer paddy raised from seeds soaked in water and dried before sowing survived better after wilting, transpired less and required less water than plants from untreated seeds. The grain yields were, however, similar under the two treatments. A morphological study by Rajagopalan (1956) did not reveal any significant difference between drought-resistant and drought-susceptible types of rice in respect of stomatal size.

JOWAR

Jowar (*Sorghum vulgare*) occupies the foremost place among the cereal crops in the scarcity tracts of the Bombay Deccan where the average rainfall varies from 20 to 27 inches (50 to 70 cm) and is largely confined to the period June to September. Jowar is grown during two seasons, (i) June to September (*Kharif*) and (ii) October to March (*Rabi*). The *rabi* crop is an important grain crop for the area and largely grows on the accumulated moisture in the soil of a tract which is a plateau with gentle undulations and therefore liable to erosion. Investigations on the water relations

of jowar, carried out by Kanitkar *et al.* (1943, 1944), are briefly reviewed here.

Water requirement: It was estimated from the average value of transpiration coefficient (424 and varying from 300 to 516) that for one ton of jowar crop (grain and straw) nearly 4.25 inches of rain was actually required. Since less than half a ton of dry matter was obtained, on an average, in this tract, it was inferred that out of an average rainfall of 26 inches hardly 4.25 inches, required for one ton, was available, the remaining 21.75 inches being dissipated by evaporation, drainage and runoff.

The transpiration coefficient was lowered, on the whole, by application of farmyard manure and/or of N,P,K. The life cycle was also shortened by application of farmyard manure.

Cultural practices: If the growth of the secondary root system were inhibited due to very dry conditions, the primary root alone could not maintain growth of the shoot for long and the seedling dried up within 3 weeks from sowing. In view of this observation, deeper sowing (about 4.5 inches as against 2.5 inches below surface) which tended to delay growth of secondary roots was not considered desirable for *rabi* sowing.

Eventually suitable soil-conservation practices and cropping patterns were worked out which are now known as the 'Bombay system of dry farming'. This method is capable of increasing the yield of *rabi* jowar by about two and a half times as compared to the yield obtained by the cultivator's method.

SUGARCANE

Water requirement: Rege *et al.* (1943) worked out suitable irrigation schedules for the Deccan Canal tract. Among four water regimes namely 70, 95, 120 and 130 acre-inches, the last one brought about an adverse effect in the later stages of growth largely due to leaching down of nitrates and suppression of microbial activity. Under this schedule the crop, however, matured earlier and the purity of juice and quality of jaggery were improved. Growth was reduced in the early stages under the 70-inch treatment, but continued longer than under 95 and 120-inch treatments and thus the difference between the final tonnages under the three treatments was negligible. The actual loss in evapo-transpiration amounted to about 67 acre-inches and as such the 70-inch treatment was likely to be insufficient in seasons of deficient rainfall. Since the 95-inch treatment made sufficient allowance for deficient rainfall it was considered to be the minimal water requirement under field conditions and 120 acre-inches were considered optimum for a manurial dose of 150 lb of nitrogen.

When water is delivered by volumetric system and is always at the disposal of a farmer, it is possible to irrigate according to requirement for growth as is done, for instance, in Hawaii. When irrigation water is available on fixed days in a month, as is the case in India, irrigation on this basis is not possible. Raheja (1944) worked out, therefore, an irrigation schedule on the basis of critical soil moisture for the North-west Frontier Province conditions (now in Pakistan). Critical limit of soil moisture was determined from the relation observed between soil moisture and

growth of the mother shoot and irrigation intervals for the early, middle and late growth stages were worked out. Thus during 7 weeks of early growth 3 irrigations were required, during the second stage the irrigation interval was 10 days and in the third stage it varied from 20 to 30 days. This irrigation schedule led to a significantly greater recovery of commercial cane sugar than what obtained under irrigation intervals of 7 or 10 days throughout the growth period.

Parthasarathy and Rao (1951) also emphasized the inverse relation between hydration of tissue and sucrose content and indicated the possibility of augmenting the recovery of sugar by adoption of agronomic practices calculated to lower hydration, under conditions obtaining in the province of Madras.

Drought resistance: Khanna and Raheja (1938, 1947), working in north Bihar where sugarcane is grown without irrigation, observed that drought resistant varieties had extensive root system, high concentration of cell sap in stalks and high content of hydrophilic colloids in leaves; these varieties also showed a quicker decline in the rate of respiration, under drought, than the susceptible varieties. Lal and Mehrotra (1949) found significant differences between 12 varieties in respect of length and breadth of epidermal cells, length and breadth of stomata and in stomatal number. Variety Rheora belonged to the group with the lowest "cell index" and because it was supposed to be drought resistant it was suggested that the lower the cell index the greater the drought resistance. It may, however, be noted that Khanna and Raheja, as well as Lal and Mehrotra, did not define the criterion of drought resistance.

WHEAT

The time of sowing of wheat in the plains of India ranges from the last week of October to the middle of November and that of harvest from mid-March to mid-April. Under unirrigated conditions the crop grows on the accumulated moisture and atmospheric and soil drought may affect the crop adversely if the weather warms up early during the period of grain-filling and if the monsoon rainfall has been short. In view of the fact that nearly two-thirds of the area under wheat is unirrigated, breeding for drought resistance was included by Pal (1944) as an item in the breeding programme initiated by him nearly 25 years ago at the Indian Agricultural Research Institute. For the purpose of selection it is desirable to have a relatively simple index of drought resistance with a sound physiological basis. Physiological investigations were conducted at this Institute by Chinoy (1947a, 1947b) from 1940 to 1947. He compared the behaviour of 260 Indian and exotic wheats of different vegetative periods and found that yield and 1000-grain weight were negatively correlated with temperature during the ripening period. It was suggested that varieties with short vegetative period should be grown where atmospheric drought set in early. Further, as the correlation of yield with temperature was practically of the same order under irrigated and unirrigated conditions and the index of soil-drought resistance (ratio of yield without irrigation to that with irrigation) differed little among varieties of

different vegetative periods, it was concluded that variability as regards response to soil drought might be very narrow and of little importance for breeding.

After Chinoy's departure from this Institute, the writer took up investigations on early and medium-early varieties. It was found as a result of investigations under pot as well as field conditions that under adequate soil moisture ear number had the most potent effect on yield, whereas under deficient soil moisture grain number per ear, and sometimes 1000-grain weight, had as much effect as ear number. Emphasis should, therefore, be laid on these two characters while selecting varieties for unirrigated conditions (Asana *et al.* 1955, Asana and Mani 1958). As emphasis in these investigations was on characters directly related to yield, further analysis of the processes concerned with grain formation was undertaken. The results are briefly outlined below.

(i) Even at the permanent wilting stage of the foliage, the water content of the ear was reduced to a very small extent.

(ii) Drought during the post-dehiscence period reduced grain number and 1000-grain weight; while grain number was reduced immediately, 1000-grain weight and grain weight per ear were not reduced until about 4 weeks after dehiscence.

(iii) During the first 4 weeks after dehiscence leaves and stem yellowed more quickly under drought, while the ear remained as green as under normal water supply.

(iv) About 4 weeks after dehiscence, 1000-grain weight and grain weight per ear increased at a slower rate under drought and the ear yellowed more rapidly. During this period leaves and stem were almost completely yellow and apparently photosynthesis in the ear controlled grain-filling (Asana, Saini and Ray 1958; Asana and Saini 1958).

Before attributing increase in grain weight to photosynthesis in the ear and the shoot, it was necessary to consider how far the loss in shoot weight, which is a normal feature during the post-dehiscence period, contributes to grain weight. From a preliminary inspection of the data obtained so far, there appears to be little consistent association between rates of loss of sugar in the stem and rate of increase of starch in the grain. Comparison of the rates of loss of sugar from stems with and without ear indicated that the rate was only slightly greater in the former; if this difference were attributed to translocation of sugar to the ear, then the latter would account for barely 10 % of the final starch content of the grain. It is, therefore, very plausible that the higher rate of grain-filling, obtaining about 4 weeks after dehiscence, under normal water supply, is largely due to slower rate of yellowing of ear. Comparison of varieties with different grain weights per ear also indicated similar trends and it may be surmised that an ear with a large number of grains and a slower rate of senescence of glumes and awns would produce better yield under unirrigated conditions.

COTTON

Sankaran (1933) compared *Gossypium herbaceum*, which is supposed to be drought resistant, with *G. indicum* and found that the former possessed a thick and deeper tap root, a denser covering of hairs on the leaves and a higher leaf water content, both under permanent wilting and normal water supply.

Dastur and Singh (1943) observed in the Punjab that heavy watering (watering at an interval of 16 to 24 hours following normal irrigation) significantly increased internodal length, boll size and yield to a small extent, but had little influence on meristematic activity. Afzal and Ahmad (1943) also noted in the Punjab a slightly beneficial effect of increased rate of irrigation on mean fibre length and percentage of mature hairs in Punjab-American cotton 4F.

CONSIDERATIONS FOR FURTHER WORK

Due to limitation of space the aspect of resistance to drought alone will be briefly referred to. For raising production of "rabi" crops, under unirrigated conditions, it is desirable to have varieties which yield better under increasing soil moisture tension. It is suggested that for discovering indices suitable for breeding such varieties, study should be concentrated in the first instance on characters which are in direct line of yield. The writer has discussed this point of view elsewhere (Asana 1957); his results on wheat have been reviewed earlier in the present article.

For a "kharif" crop like rice, which is grown during the monsoon period (it is also a "rabi" crop in several tracts), moisture deficit may occur under upland conditions. In view of the observations of Takeda and Maruta (1957) that photosynthesis in the rice plant, during the ripening period, plays an important role in grain production, it would seem profitable to attempt assessment of drought resistance in rice on the lines followed by the writer for wheat.

In north Bihar, where sugarcane is grown without irrigation, the writer (1950) observed that drought affected tonnage by depressing the rates of net assimilation and leaf and internode expansion. It is suggested that these attributes may be considered for the assessment of drought resistance of sugarcane varieties.

The "kharif" crops are just as likely to be subjected, during monsoon, to waterlogging as to deficient soil moisture. Lundkwist (quoted by Stocker 1959) observed that deficient as well as excessive soil moisture tended to bring about more or less similar morphogenetic effects, such as increase in frequency of stomata per unit area and reduction in length of guard cells. The writer (1950) also observed under north-Bihar conditions that growth of sugarcane under low-lying water-logged conditions was checked due to depression in rate of leaf growth and uptake of nitrogen; the apparent similarity of the effect of deficient and excessive soil moisture on leaf growth may be noted. As such it would be worthwhile to inquire how far a correlation between the effects of deficient and excessive soil moisture obtains in the case of some important yield-determining characters of "kharif" crops and to investigate the mechanism underlying these effects.

REFERENCES

Afzal, M. and Ahmad, N., 1943, Effect of different irrigations on field behaviour and quality of Punjab-American 4F cotton, *Ind. J. agric. Sci.*, **13**, 357–67.

Asana, R. D., 1950, Growth analysis of the sugarcane crop in North Bihar (India). I. Seasonal variation in growth and yield in unmanured plots, *Ann. Bot. N. S.*, **14**, 465–86.

Asana, R. D., Mani, V. S., Pillay, K. P. and Gahlot, K. N. S., 1955, Analysis of drought resistance in crop plants. I. The influence of soil drought on the relation between yield and ear characters in wheat (pot culture), *Ind. J. Genet. & Pl. Breed.*, **15**, 59–79.

Asana, R. D., Mani, V. S., Pillay, K. P. and Gahlot, K. N. S., 1957, The problem of assessment of drought resistance in crop plants, *ibid.*, **17**, 370–78.

Asana, R. D., Mani, V. S., Pillay, K. P. and Gahlot, K. N. S., 1958, Analysis of drought resistance in crop plants. II, *ibid.*, **18**, 187–98.

Asana, R. D. and Saini, A. D., 1958, Studies in physiological analysis of yield. IV, *Physiol. Plant.*, **11**, 666–74.

Asana, R. D., Saini, A. D. and Ray, D., 1958, Studies in physiological analysis of yield. III. The rate of grain development in wheat in relation to photosynthetic surface and soil moisture, *Physiol. Plant.*, **11**, 655–65.

Chinoy, J. J., 1974a, Correlation between yield of wheat and temperature during ripening of grain, *Nature*, **159**, 442–44.

Chinoy, J. J., 1947b, How drought affects Indian wheats, *Ind. Farming*, **8**, 72–74.

Dastur, R. H., 1924, Water content a factor in photosynthesis, *Ann. Bot.*, **38**, 779–88. (Also, *ibid.* **39**, 769; **47**, 69).

Dastur, R. H. and Singh, M., 1943, Studies in the periodic partial failures of the Punjab-American cottons in the Punjab. IX, *Ind. J. agric. Sci.*, **13**, 610–30.

Ghose, R. L. M., Ghatge, M. B. and Subrahmanyan, V., 1956, Rice in India, *Ind. Coun. agric. Res. Publ.*, New Delhi.

Ghosh, B. N., 1949, Physiological studies on the effect of varying water levels on growth of rice in relation to carbohydrate metabolism of the leaves, *Bull. bot. Soc. Bengal*, **3**, 1–8.

Ghosh, B. N., 1954, Studies in the physiology of rice. VII. Effect of varying water levels on growth of rice in relation to nitrogen absorption, *Proc. nat. Inst. Sci. India*, **20**, 371–87.

Ghosh, B. N., 1959, A preliminary study of the effect of different levels of water on growth and yield of spring (BORO) paddy, *Sci. and Cult.*, **24**, 387–89.

Inamdar, R. S. and Dabral, B. M., 1930, Experiments on transpiration. I. The daily water balance of plants in arid regions, *J. Ind. bot. Soc.*, **9**, 17.

Kanitkar, N. V., Gode, R. B. and Gokhale, D. H., 1943, Water requirement of rabi jowar in the scarcity tracts of the Bombay Deccan, *Ind. J. agric. Sci.*, **13**, 235–51.

Kanitkar, N. V., Gode, R. B. and Gokhale, D. H., 1944, Dry farming in India, *Ind. Coun. agric. Res.*, Monograph No. **15**, New Delhi.

Khanna, K. L. and Raheja, P. C., 1938, Preliminary studies in the respiration of sugarcane in situ, *Ind. J. agric. Sci.*, **8**, 253–69.

Khanna, K. L. and Raheja, P. C., 1947, Some observations on the water relations of sugarcane plant in North Bihar, *ibid.*, **17**, 353–69.

Lal, K. N. and mehrotra, O. N., 1949, Studies in crop physiology. Cell size characteristics of sugarcane varieties in relation to drought resistance, *Bot. Gaz.*, **111**, 193–210.

Leather, J. W., 1910, Water requirements of crops in India, *Memoirs Dept. agric. India, chem. Ser.*, **1**, 133–84.

Leather, J. W., 1911, Water requirements of crops in India, *ibid.*, **2**, 205–81.

Pal, B. P., 1944, The Pusa wheats. The wheat breeding work at the Imperial Agricultural Research Institute, *Emp. J. exp. Agric.*, **12**, 61–73.

Parija, P. and Pillay, K. P., 1944, Inducing flood resistance in paddy by pre-sowing treatments, *Curr. Sci.*, **13**, 183–85.

Parija, P. and Pillay, K. P. 1945, Effect of pre-sowing treatment on the drought resistance in rice, *Proc. nat. Acad. Sci. India*, **15**, 6–14.

Parthasarathy, S. V. and Rao, R. M. V., 1951, Tissue moisture and its relation to sugarcane ripening, *Proc. 1st bienn. Conf. Sugarcane Res. Workers, India, bot. Sec.*, 5–12.

RAHEJA, P. C., 1944, Relative irrigation requirements of sugarcane, *Proc. nat. Inst. Sci. India*, **10**, 289–300.

RAJAGOPALAN, K., 1956, Variability in size and frequency of stomata in leaves of rice varieties and its correlation to drought resistance, *Madras agric. J.*, **43**, 429–43.

RAMDAS, L. A., 1957, Evaporation and potential evapo-transpiration over the Indian sub-continent, *Ind. J. agric. Sci.*, **27**, 137–49.

REGE, R. D. ET AL., 1943, Problems of sugarcane physiology in the Deccan canal tract. V. Water requirement, *ibid.*, **13**, 413–33.

SANKARAN, R., 1933, Some aspects of drought resistance with special reference to cotton, *Read at the Assoc. econ. Bot.*, Coimbatore.

SEN, P. K., 1937, Studies in water relations of rice. I. Effect of watering on the rate of growth and yield of four varieties of rice, *Ind. J. agric. Sci.*, **7**, 89–117.

SINGH, B. N. ET AL., 1935, Investigations into the water requirements of crop plants, *Proc. Ind. Acad. Sci.*, **1(B)**, 47–95.

SINGH, B. N. AND MEHTA, B. K., 1938, Water requirement of wheat as influenced by the fertility of the soil, *J. Am. Soc. Agron.*, **30**, 395–98.

SINGH, B. N. AND SINGH, B. R., 1936, Growth and water requirement of crop plants in relation to soil moisture, *Proc. Ind. Acad. Sci.*, **4 (B)**, 376–402.

SINGH, B. N. AND SINGH, J. R., 1939, Effectiveness of chemical fertilizers on the growth and water requirement of wheat, *Proc. Ind. Acad. Sci.*, **10(B)**, 27–40.

STOCKER, O., 1959, Physiological and morphological changes in plants due to water deficiency. UNESCO'NS'AZ'414 Public.

TAKEDA, T. AND MARUTA, H., 1957, Studies on CO_2 exchange in crop plants. Roles played by various parts of the photosynthetic organs of the plant in producing grains during the ripening period, *Field Crop Abst.* **10** (Feb.), item 119.

THE DIAGNOSIS AND CONTROL OF ZINC DEFICIENCY AND EXCESS*

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ABSTRACT

Visual symptomatology, and leaf and soil analysis values associated with zinc deficiency and excess, together with examples of control methods for a variety of crops are brought together in this paper.

Although there are still many blind spots in existing information, enough data are at hand to provide effective guidance in determining the zinc status of a wide variety of plants, and suitable methods of correcting both zinc deficiency and zinc excess have been worked out.

With most crops, zinc values in the dry matter of leaves under 20 ppm can be regarded as indicating either deficiency or approaching deficiency.

Values over 20 ppm and less than 200 ppm can generally be regarded as indicating ample but not excessive zinc. Though sufficient data are not a hand, values over 300 ppm zinc in the dry matter of well-cleaned leaves may indicate a condition of excess or one of approaching excess.

When supplemental information is needed, soil analysis by any one of the several availability methods described will provide additional confirming evidence.

Rapid control of zinc deficiency in most all crops can be achieved by sprays. Those applied to foliage usually depend on zinc sulfate safened with half of its weight of lime hydrate. Soil application methods are also effective in many instances, but are not as well worked out for a wide variety of crops, soils, and climates as spray methods.

INTRODUCTION

The enormous increase in criteria for diagnosing nutrient status (visual symptoms, leaf and soil analyses, knowledge of soils and plants) during the past 30 years means that no longer does the farmer need to depend upon rule-of-thumb fertilizer practices or be guided solely by the results of often inadequate fertilizer trials and general experience.

Unfortunately, much of the pertinent information available is widely scattered and though good summeries of visual symptomology [e.g., Wallace (1953), and plant analysis values (Goodall & Gregory 1947, and Childers 1954)] have been prepared, no one (to the knowledge of the author) has ever brought together in one publication all of the information, both plant and soil, useful in evaluating nutritional status with respect to a given element, and summarized information on control methods.

This paper represents such an attempt for the element zinc. No doubt, much additional unpublished information exists, and probably the author has missed some published data.

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Similar information is being assembled for all of the other elements. When this is completed and various summary tables prepared, the material will be issued as a handbook of diagnostic criteria.

It will be evident, from a brief perusal of the data assembled herein, that despite the substantial amount of research devoted to one or another aspect of zinc nutrition during the past 30 years, there is still need for more information, This is particularly true as regards leaf and soil analysis standards. Visual symptomatology is adequate on many crops, but incomplete or lacking for others.

HISTORICAL

The essentiality of zinc for plant life was not fully accepted until the early 1930's, when Chandler *et al.* (1932), working with peaches, and Johnston (1933) and Parker (1934), working with citrus, and others, were able to correct little leaf of peaches and mottle leaf of citrus, respectively, with zinc compounds. Following this, Hoagland *et al.* (1936, 1937) and Chapman *et al.* (1937) were able to produce in controlled cultures the self-same field symptoms correctable by zinc. It was during this period that more general acceptance of the early work of Raulin (1863, 1869, 1870), Javillier (1908, 1912, 1914a, 1914b), Steinberg (1918, 1919), Maze (1914, 1915), Sommer and Lipman (1926), and Sommer (1927, 1928) came into clear focus and investigators generally accepted that zinc was an essential plant food element. Since this time, zinc deficiency has been found on many soil throughout the world, and new cases are being brought to light every year. A good summary entitled 'Zinc deficiency and its control' has recently been published by Thorne (1957).

Zinc toxicity was clearly recognized early and many reports of its toxicity were summarized by Brenchley (1927). Numerous others since have noted zinc toxicity both in the field and under controlled culture conditions.

A substantial amount of literature concerned with the visual symptoms of deficiency and excess, the zinc content of leaves and other plant parts under deficiency and excess conditions, the zinc content of soils and tests for availability, control methods, as well as physiological function and soil chemistry, has appeared in the past 30 years. Information having to do with diagnosis and control is presented in largely tabular form in the pages which follow to provide guidance with respect to evaluating zinc status and controlling zinc deficiency and excess.

PLANT CRITERIA

Growth characteristic and leaf symptomatology of acute zinc deficiency is, with many crops, so well defined that supplementary leaf and/or soil analyses tests are not necessary. However, the early stages with many crops cannot be readily identified and leaf analyses and/or soil analyses will provide information of great value. Excess zinc often produces iron chlorosis.

VISUAL SYMPTOMS OF ZINC DEFICIENCY AND EXCESS

Zinc deficiency

Early stages: With citrus, it is common to see a few leaves with the typical symptoms of 'mottle leaf' or 'frenching', as it is commonly called, long before the more acute stages characterized by small leaves and dieback appear.

Moderate to acute stages: In fruit trees, small leaves with a rosette-type terminal growth are common. Citrus shows a typical interveinal chlorosis termed 'mottle leaf', or by some 'frenching' (Figure 1 and 2). In most plants, the terminal growth is first affected and there is a decrease in stem length and a rosetting or whorling of leaves. In corn, there is a white striping of leaves (Figure 3). For a more detailed description on various crops, see the section which follows.

Specific symptomatology of selected crops (Enlarged from Chapter 8 in *Diagnostic Techniques for Soils and Crops*, by J. E. McMurtrey, Jr., 1948. Published by American Potash Institute)

Plant	Visual Symptoms	Reference
Apple (*Malus sylvestris*)	Leaves developing in spring characterized by whorls of small, stiff, and sometimes mottled leaves near tips of current season's growth; except for terminal rosettes, twigs are bare for some time; later, branches may arise below twigs and produce almost normal leaves early, but later become mottled and misshapen; fruit bud formation reduced; developing fruits small and malformed; twigs may die back after first year.	Davidson and Judkins (1949)
Barley (*Hordeum* sp.)	Leaves show uniform chlorosis, drying up; tip growth ceases.	Sommer (1928)
Broad bean (*Vicia faba*)	Leaves and flower buds shed; seed pods fail to develop.	Sommer (1928)
Corn (*Zea mays*)	Older leaves have light yellow streaks between veins, followed rapidly by necrosis; this condition may become so extreme that young leaves unfolding in bud are white to light yellow.	Barnette *et al.* (1935, 1936)
Coffee (*Coffea arabica*)	One or more branches have short internodes, reduction in leaf and fruit size, and chlorosis. Later may resemble "mottle leaf" of citrus pattern. Dieback occurs.	Franco and Mendes (1954)
Cotton (*Gossypium* sp.)	Leaves chlorotic with necrotic areas.	Hoagland *et al.* (1936)
Flax (*Linum usitatissimum*)	Grayish brown collapsed spots appear in youngish leaves, followed by drying and color changes to brown or white. Internodes between leaves are shortened with rosetting appearances. Later, top of main stem becomes necrotic.	Millikan (1942)
Garden bean (*Phaseolus vulgaris*)	Leaves and flower buds shed.	Sommer (1928)
Garden pea (*Pisum sativum*)	Lower leaves necrotic at margins and tips; stems stiff and erect; flowers none.	Piper (1940)

Plant	Visual Symptoms	Reference
Grapefruit (*Citrus paradisi*)	Leaves chlorotic, known as "mottle-leaf" in California and "frenching" in Florida; irregular green bands along midribs and lateral veins; twigs tend to die back; fruit quality and quantity vary with severity of deficiency.	Camp *et al.* (1949)
Lemon (*Citrus limon*)	*See* Grapefruit.	Camp *et al.* (1949)
Oat (*Avena sativa*)	Leaves pale green; older leaves show collapsed areas at margins and tips, grayish in color, necrosis extends down leaf, remainder of leaf grey to bronze-green.	Piper (1940)
Orange (*Citrus sinensis*)	*See* Grapefruit.	Camp *et al.* (1949)
Peach (*Prunus persica*)	Leaves chlorotic, mottling progressing upward from lowermost leaves; leaves narrow, more or less crinkled; twigs short, internodes near tips producing rosettes of leaves; defoliation progressing upward; fruit bud formation drastically curtailed; fruits few, misshapen, and worthless.	Nelson and Bear (1949)
Pecan (*Carya illinoensis*)	Leaves chlorotic as a yellow mottling between veins, regions along margins and veins may remain green; sometimes only one or two branches affected; later leaves may show bunching due to shortened axes at twig ends, "pecan rosette."	Alben *et al.* (1932)
Potato (*Solanum tuberosum*)	Leaves with grayish brown to bronze irregular spots, usually on leaves halfway up plant, but sometimes on older or younger leaves and finally on almost all leaves; with severe deficiency, stems and leaf petioles develop brown spots; plants short.	Jones *et al.* (1949)
Squash (*Cucurbita maxima*)	Leaves mottled with necrotic areas.	Hoagland *et al.* (1936)
Sugar beet (*Beta vulgaris*)	Leaves necrotic, develop brown to grayish spots; tops of leaves wither; only petioles retain green colour.	Piper (1940); van Schreven (1937)
Tangerine (*Citrus nobilis* var. *deliciosa*)	*See* Grapefruit.	Camp *et al.* (1949)
Tobacco (*Nicotiana tabacum*)	Lower leaves show slight chlorosis at tips and margins, followed by necrosis of chlorotic tissue, small areas at first, sometimes surrounded by halo, these areas rapidly enlarge, become water-soaked in appearance; small veins are at first involved in breakdown, but, later, veins as well as tissue between veins dry up; internodes short, leaves apparently thickened.	McMurtrey (1949)
Tomato (*Lycopersicon esculentum*)	Leaves mottled and necrotic, leaflets small, midrib shortened	Hoagland and Arnon (1938)
Tung (*Aleurites fordi*)	Leaf bronzing, necrosis, and spotting produce ragged appearance followed by defoliation, rosetting, or bunching of new leaves.	Mowry and Camp (1934)

Figure 1a →
Ioderately acute "mottle eaf" pattern on orange shoot,

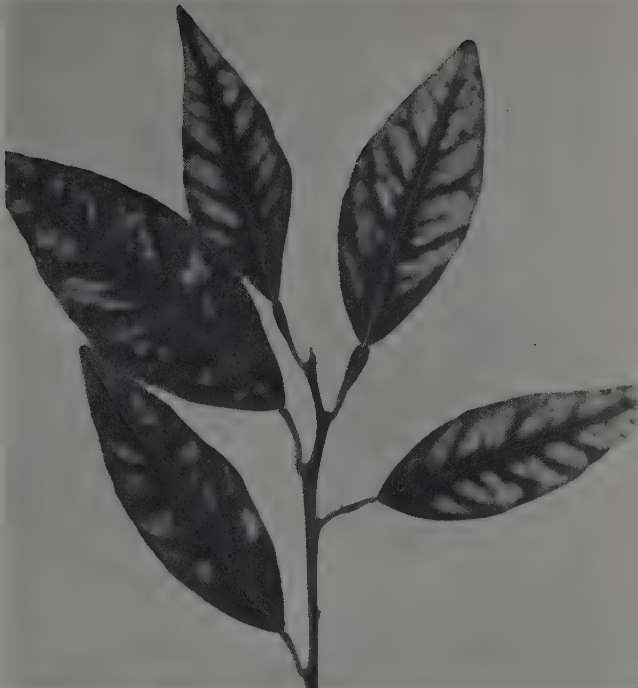

Figure 1b ↓
cute stage characterized y very small chlorotic leaves, ieback, and shortened inter-odes.

Figure 1

Figure 2
Zinc deficiency patterns on avocado leaves. (Photo by E. F. Wallihan).

Figure 3
Effects of zinc deficiency on young corn plants. Chlorotic stripes in mid-section of leaves is the most noticeable characteristic. (Photo supplied by Frank G. Viets, Jr).

Zinc excess

Early stages: No defining information.

Moderate to acute stages: Excess zinc commonly produces iron chlorosis in plants: Chapman *et al.* (1939), Smith and Specht (1953), Hewitt (1948), and Hunter and Vergnano (1953).

Specific symptomatology of selected crops

Citrus	Leaves show iron chlorosis.	Chapman *et al.* (1939)
Barley and grasses	Rusty brown flecks on leaves and death.	Brenchley (1927)
Oats	Leaves show iron chlorosis.	Hunter and Vergnano (1953)
Sugar beet	Leaves show iron chlorosis.	Hewitt (1948)

INDICATOR PLANTS

Zinc deficiency

Fruit trees, particularly citrus and peaches, are good indicators and commonly show zinc deficiencies in soils where field and garden crops grow normally.

Corn (*Zea mays*) is one of the field crop plants most sensitive to lack of zinc. Oats, wheat, barley, and rye are insensitive to zinc supply, according to Viets, Boawn, and Crawford (1954).

Zinc excess

According to Robinson *et al.* (1947), a good indication of a zinc mineral outcrop is the presence of luxuriantly growing ragweed when other vegetation is stunted. He studied the distribution and growth of 30 plant species growing on former zinc slime ponds, where the total zinc of the soil was 12.5%. Zinc content ranged from 39 ppm in the fruit of False Solomon's Seal to 5400 ppm in horsetail.

TISSUE ANALYSIS VALUES

According to Holmes (1944), plants of different kinds will vary from a minimum of 20 ppm to 10,200 ppm zinc. Actually, many plants show values under 20 ppm. There is much research to show that zinc readily accumulates in the leaves of many plants in luxury amounts. This great spread in the zinc content of leaves, where deficiency is characterized by very low values and excess by high values, provides the basic reason for believing that the zinc content of leaves of known age provides a reasonably sound basis for evaluating zinc status. The data in Table I that follows shows that in a wide variety of plants deficiency levels are characterized by zinc levels of less than 20 to 25 ppm in the dry matter. Ample but not excessive levels commonly fall in the range of 25 to 150 ppm. Much less data on excess levels exist, but the information at hand indicates that amounts in excess of 400 ppm are suggestive of zinc excess. Much more information on what constitutes threshold values of zinc excess is needed.

TABLE I

Tissue analysis values useful in indicating zinc status (Adapted and broadened from Goodall and Gregory 1947)

Plant species	Type culture	Tissue sampled	Age, stage, or date of sample	Range in dry matter (ppm)					Reference
				Showing deficiency symptoms	Low range	Without symptoms	High range	Showing toxicity sypmtoms	
Alfalfa (*Medicago sativa*)	Field	Top ½ of shoots	Bloom stage	8.0	—	13.8	—	—	Boawn & Viets (1952)
	Controlled	Tops	12 weeks old	13.0	—	39.0–48.0	—	—	Millikan (1953)
Apple (*Malus sylvestris*)	Field	Lvs. of apical 6–8"	Sept.–Oct.	4.0–54.0	—	4.0–80.0	—	—	Chandler *et al.* (1934)
	Field	Stems of apical 6–8"	Sept.–Oct.	4.0–28.0	—	16.0–80.0	—	—	Chandler *et al.* (1934)
	Field	Leaves	—	5.0–10.0	—	—	—	—	Bould *et al.* (1953a, 1953b)
	Field	Leaves	—	1.2–2.3	—	9.6–10.8	—	—	Bould *et al.* (1949)
	Field	Leaves	August	< 14.0	—	—	—	—	Thomas *et al.*(1954)
	Field	Leaves	—	3.0–22.0	—	6.0–40.0	—	—	Woodbridge(1951)

Apricot (*Prunus armeniaca*)	Field	Lvs. of apical 6–8"	Sept.–Oct.	24.0–30.0	—	19.0–31.0	—	—	Chandler *et al.* (1934)
	Field	Stems of apical 6–8"	Sept.–Oct.	7.0–9.0	—	11.0–34.0	—	—	Chandler *et al.* (1934)
Avocado (*Persea americana*)	Field	Leaves	Mature	4.0–15.0	—	50.0	—	—	Wallihan *et al.* (1958)
Citrus (*Citrus aurantium, sinensis*, etc.)	Field	Leaves	—	3.8–11.7	—	7.8–47.3	—	—	Gaddum *et al.* (1936)
	Field	Leaves	3–12 months	4.0–15.0	—	20.0–80.0	—	—	Thomas & Laurance (1933)
	Field	Leaves	—	4.0–6.0	—	8.0–10.0	—	—	McGeorge (1939)
	Field	Leaves	—	10.0–26.0	—	14.0–34.0	—	—	McGeorge (1949)
	Field	Leaves	5 months	13.0–21.0	—	22.0–46.0	—	—	Reuther & Smith (unpubl.)
	Field	Leaves	4–7 months	<15.0	16.0–24.0	25.0–100.0?	110.0–200.0?	300.0?	Reuther & Smith (1954)
	Field	Leaves	4–7 months	15.0	—	20.0–80.0	—	—	Chapman (1949)
	Field	Leaves	—	7.1	—	—	—	—	Healy (1952)

Continued on next page

TABLE I (Continued)

Tissue analysis values useful in indicating zinc status

Plant species	Type culture	Tissue sampled	Age, stage, or date of sample	Range in dry matter (ppm)					Reference
				Showing deficiency symptoms	Low range	Without symptoms	High range	Showing toxicity symptoms	
Corn (*Zea mays*)	Field	Lower leaves	Tasseling	9.0–9.3	—	31.1–36.6	—	—	Viets *et al.* (1953)
	Field	Lvs. from 6th node from base	Silking	14.6–15.1	—	>15.0	—	—	Viets *et al.* (1953)
Flax (*Linum usitatissimum*)	Pot	Tops	71 days old	18.0	—	32.0–83.0	—	—	Loneragan (1951)
Oats (*Avena sativa*)	Sand	Leaves	Mature	—	—	—	—	1700.0–7500.0	Hunter & Vergnano (1953)
	Sand	Whole plant	Prior to inflorescence exsertion	>20.0	—	—	—	—	Wood & Sibly (1950)
Peach *Prunus persica*)	Field	Leaves	Apical 6–8" Sept.–Oct.	6.0–15.0	—	6.0–43.0	—	—	Chandler *et al.* (1934)
	Field	Stems	Apical 6–8" Sept.–Oct.	5.0–12.0	—	11.0–50.0	—	—	Chandler *et al.* (1934)
	Field	Leaves	From medium shoot positions; August sampling	3.5–25.4	—	6.0–140.0	—	—	McClung (1954)

Continued on next page

Pear (*Pyrus communis*, etc.)	Field	Leaves	Lower part Current season	9.9	—	16.0	—	—	Bollard (1953)
Pecan (*Carya*	Field	Leaflets	August	3.7	—	3.9–16.7	—	—	Finch & Kinnison (1933)
illinoensis)	Field	Leaves	Top of tree set	Trace–7.0	—	66.0–202.0	—	—	Finch (1936)
	Field	Petioles	August	Trace	—	Trace–10.0	—	—	Finch & Kinnison (1933)
	Field	Shoots	August	Trace	—	7.9–15.3	—	—	Finch & Kinnison (1933)
Pineapple (*Ananas sativus*)	Field	Leaves	Base	4.0–26.0	—	16.0–44.0	—	—	Lyman & Dean (1942)
	Field	Leaves	Distal end	4.0–26.0	—	4.0–22.0	—	—	Lyman & Dean (1942)
	Field	Stem	Growing point	6.0–96.0	—	144.0–158.0	—	—	Lyman & Dean (1942)

Continued on next page

TABLE I (Continued)

Tissue analysis values useful in indicating zinc status

Plant species	Type culture	Tissue sampled	Age, stage, or date of sample	Range in dry matter (ppm) Showing deficiency symptoms	Low range	Without symptoms	High range	Showing toxicity symptoms	Reference
Potato (*Solanum tuberosum*)	Field	Leaves	Mature mid-section	—	>30.0	30.0–87.0	—	—	Klostermann *et al.* (1956)
Subterranean clover (*Trifolium subterraneum*)	Field	Leaves	Flowering	>15.0	—	—	—	—	Teakle & Turton (1943)
	Solution	Tops	12 weeks old	24.0–25.0	—	76.0–90.0	—	—	Millikan (1953)
Tomato (*Lycopersicon esculentum*)	Field	Leaves	Mid-season	6.0–8.7	—	13.0	—	—	Lingle *et al.* (1958)
	Sand	Leaves	Middle $^1/_3$ of plant	14.4	—	26.9	—	—	Lyon *et al.* (1943)
	Solution	Leaves	Basal & medium lvs. at fruit setting stage	9.0–15.0	—	65.0–198.0	—	526.0–1489.0	Lyon *et al.* (1943)
Tung (*Aleurites* spp.)	Field	Leaves	—	3.6–6.2	—	15.7–35.8	—	—	Gaddum *et al.* (1936)
	Field	Petioles	—	3.7–4.0	—	9.1–28.4	—	—	Gaddum *et al.* (1936)
	Field	Leaves	Mid-shoot	10.0–26.0	—	30.0–229.0	—	—	Drosdoff (1950)
	Sand	Leaves	Mid-shoot, Aug.	—	—	—	—	485.0	Shear (1958)
Walnut (*Juglans regia*)	Field	Leaves	Apical 6–8"	11.0–22.0	—	16.0–30.0	—	—	Chandler *et al.* (1934)
	Field	Stems	Apical 6–8"	8.0–17.0	—	24.0–34.0	—	—	Chandler *et al.* (1934)

SOIL CRITERIA

Zinc deficiency

Kinds of soil in which zinc deficiency most commonly occurs

1. Acid, leached, sandy soils where total zinc is low.
2. Alkaline soils where zinc availability is decreased.
3. Soils derived from granites, gneisses, etc.
4. Old corral sites and indian burying grounds; Chandler *et al.* (1946).
5. Some organic soils where zinc is tied up in forms difficultly available to plants.
6. Soils containing clays with low Si/Mg ratios. In these; zinc may be fixed in forms not readily available to plants; Elgabaly (1950).

Soil management and other practices which aggravate or reduce zinc deficiency

1. High phosphate soils. Heavy dosages or prolonged use of phosphate fertilizers have been shown on many soils to decrease zinc uptake and even bring on zinc deficiency of various crops; West (1938); Reuther & Crawford (1946); Bingham & Martin (1956); Mowry & Camp (1934); Chapman, Liebig & Vanselow (1939); Thorne & Wann (1950); Winters & Parks (1955); Labanauskas, Embleton & Jones (1958); Loneragan (1951); Millikan (1947a); Rogers & Wu (1948); Leggett (1952); and Bathurst (1945).
2. Liming acid (esp. sandy) soils may induce zinc deficiency; Lott (1938); Wear (1956). Minimum zinc solubility is in the range *p*H 6.0 to 8.0, according to Jurinak & Thorne (1955).
3. Nitrogen fertilization often increases the severity of zinc deficiency; Ozanne (1955); Chapman *et al.* (1937); Camp (1945); Reuther & Smith (1950); Haas (1936). This may be related to associated soil *p*H changes or to cation effects rather than to nitrogen itself. Viets *et al.* (1957) found that $NaNO_3$ decreases whereas NH_4NO_3 and $(NH_4)_2SO_4$ increases zinc uptake.
4. Alfalfa cover crops in orchards tend to reduce or prevent zinc deficiency. This is thought due in part to roots bringing zinc to the soil surface, followed by top decomposition and release. Other mechanisms may be involved, too. For example, Millikan (1953) suggested that alfalfa roots may solubilize zinc and help other plants obtain more.
5. Soil fumigation or sterilization prior to planting a crop frequently improves its zinc-supplying power or availability, or perhaps the explanation is that by reducing detrimental organisms roots grow and forage better. This has been noted in new citrus plantings on old citrus soils where comparisons of fumigation and non-fumigation have been made.
6. Under soil conditions where there has been long continued built-up of organic matter in surface layers, zinc accumulation is favoured; Hibbard (1940b). Apparently zinc is brought up from lower horizons by plant roots and is held in the resultant organic residue. Much of this zinc is in available form.

7. Additions of organic matter to soil or growing green manure crops frequently improves crops subject to zinc deficiency. Skinner and Demaree (1926) noted this with pecan rosette even before the true nature of this disorder was known. Briggs, Jensen and McLane (1916) made similar observations on mottle leaf of citrus, noting an inverse correlation between humus content of soil and mottle leaf.

Zinc excess

Kinds of soil in which zinc excess occurs

1. Acid peats of some kinds; Walsh and Clarke (1945).
2. In vicinity of lead mine dumps where soil has been contaminated with seepage; Davies (1941).
3. Soil contaminated with zinc from mining operations; Robinson *et al.* (1947).
4. Soil derived from rocks and materials high in zinc; Swaine (1955).

Soil management and other practices which may produce zinc toxicity

1. Acidification of some soils.
2. Accumulation of excess zinc from zinc fertilization. This has occurred on some Florida soils and will be an increasing hazard with the more widespread use of zinc in sprays or fertilizers. It may become especially hazardous where such soils become acid as a result of the use of acid-forming fertilizers.

SOIL ANALYSIS VALUES OF SIGNIFICANCE

Zinc deficiency

Total zinc: Total zinc in soils varies from 10 to 300 ppm, according to Swaine (1955). It is usually more concentrated in surface than sub-surface horizons; Hibbard (1940a); Thorne *et al.* (1942).

Alben and Boggs (1936) found rosette of pecan trees (zinc deficiency) on acid soils where the total zinc in the root zone was from 116 to 136 pounds per acre.

Thorne, Laws and Wallace (1942) found that total zinc differentiated zinc-deficient from non-zinc-deficient soils as well as the Hibbard's extraction procedure.

Extractable zinc: Bergh (1947a, 1947b, 1948) extracted 20 to 30 g soil by shaking for 1 hour with 200 to 300 ml 0.1N $MgSO_4$ with the *p*H of the extracting solution adjusted to that of the original soil. Zinc deficiency of wheat and barley occurred when zinc values of the soil were 3.5 and 6.6 ppm, respectively. Highest yield of wheat occurred when extractable zinc was 160 ppm; rye, 40; barley, 95; and oats, 480.

Shaw and Dean (1952) extracted 2.5 g soil with 50 ml of an ammonium acetate-dithizone-carbon tetrachloride reagent. Where Zn values of 1 ppm and less on soils of *p*H 7 or higher were found, many crops showed Zn deficiency. With soils of *p*H 6 and lower, 2.5 ppm Zn was found ample.

Conner (1920) extracted some acid soils which had been in contact with galvanized pots with cold 1N KNO_3. These soils showed zinc ranging from 880 to 2130 ppm, and these amounts were sufficient to be toxic to wheat and clover.

Lyman and Dean (1942) extracted the 0–12" layer of a number of Hawaiian pineapple soils with ammonium acetate at pH 4.6, and where zinc deficiency of pineapples was acute got values of from 0.5 to 0.6 ppm Zn as against 1.7 to 3.5 ppm where no zinc deficiency occurred.

Massey (1957), working with 34 silt loam soils of Kentucky, found that zinc uptake by corn in pots was related to pH and to the amount of zinc extracted by Shaw and Dean's ammonium acetate-dithizone-carbon tetrachloride reagent. He proposed the formula $Y = 99.2 - 12.2x_1 + 10.9x_2$. Where x_1 is pH and x_2 the dithizone extractable zinc in ppm dry soil and Y the calculated zinc uptake. Where the value of Y is greater than 40, no zinc deficiency can be looked for, and when it is lower the chances are high that reponses to zinc will be obtained.

Tucker and Kurtz (1955) found that the amounts of zinc removed by successive extractions with 0.1N HCl (using a 1:10 soil water ratio and a 45-minute extraction period) over a 6-week period gave essentially the same value as the zinc removed by the *Aspergillus niger* bio-assay method. No correlative data relating 0.1N HCl soluble Zn to crop performance is given, but the soil values found for the chemical method ranged from 2.2 to 3.3 ppm zinc in the soil, and no zinc responses were obtained on these soils.

Wear and Sommer (1947), working with Albama soils, found a good correlation between the amounts of zinc extracted by 0.1N HCl (1:10 soil extractant) and the presence or absence of deficiency symptoms. Where deficiency symptoms occurred, the zinc content ranged from 0.50 to 0.90 ppm. Where no deficiency symptoms occurred, the values ranged from 1.20 to 4.70 ppm zinc in the soil. The soils in question had pH values ranging from 4.7 to 6.2.

Aspergillus niger test: Bould *et al.* (1953a, 1953b) found zinc deficiency of apples and pears growing on soils containing less than 2 ppm of zinc removable by *A. niger*. Normal soils contained about 10 ppm.

Tucker, Kurtz and Lynch (1953) found 0.6 to 2.88 ppm zinc on Florida soils known to respond to zinc. Relative growth of *A. niger* in a culture medium to which standard amounts of soil are added as compared with growth from the addition of increasing increments of zinc is the method by which the zinc-supplying power of the soil is determined.

Viets, Boawn and Crawford (1954) found 0.80 to 1.3 ppm Zn extractable by 0.1N HCl from soils where various field crops show zinc deficiency as against 1.3 to 1.8 ppm in soils where no deficiencies occur.

Zinc excess

Barnette (1936) found that 400 ppm zinc in replaceable form was toxic to corn. Calcium carbonate at 4,000 lbs./acre overcame the toxicity.

Gall (1936) found that 500 lbs./acre of Zn as zinc sulfate was markedly toxic to cowpeas; 700 lbs./acre was toxic to corn. Trials were in pots ot typical Florida sands.

Gall and Barnette (1940) in pot culture tests found that 0.688 to 1.376 milliequivalents Zn per 100 g were toxic to corn on a Norfolk sand; 0.758 to 1.137 me on an Orangeburg fine sandy loam, and between 1.615 and 2.153 me on a Greenville clay loam. Cowpeas were affected on a Norfolk sand at 0.275 to 0.482 me Zn/100 g. Calcium carbonate at 1,000 lbs. per acre markedly decreased the zinc toxicity at these concentrations.

Staker and Cummings (1941) and Staker (1942) found some New York peat soils where zinc is present in toxic concentration to vary from 0.43 to 10.16% zinc. Most of this zinc was found to be held in exchangeable form. Vegetable crops, such as spinach, lettuce, and carrots, were seriously affected. Application of sufficient sodium hydroxide or calcium hydrate to bring the *p*H to neutrality reduced the toxicity in those soils which were not too high in zinc.

Hawkins and Cameron (1953) found that some tree seedlings grown in galvanized iron tubes of soil were stunted, yellowed, and in some cases killed as a result of the roots coming in contact with the zinc surface.

Hewitt (1948) produced iron chlorosis in sugar beets grown in sand when 0.5 and 1.0 milliequivalents per liter of Zn^{++} were added to the nutrient solution. This occurred both when iron was supplied as the citrate and when supplied as magnetite incorporated in the sand. Other metals also produced iron chlorosis under the same conditions.

Hunter and Vergnano (1953) produced iron chlorosis in oats grown in sand culture when zinc was added to the culture solution. At 25 ppm added zinc, the chlorosis was faint and the oat leaves contained 1,700 ppm Zn. When 100 ppm Zn was added to the culture solution, the chlorosis was more severe and the leaves showed 7,500 ppm.

Millikan (1947b) produced iron deficiency in flax in nutrient solutions by adding zinc as $ZnSO_4.7H_2O$ at rates to give 2.5, 5.0, 10.0, 20 and 50 ppm. Additions of molybdenum to the solution reduced the toxicity. It required 5 to 25 ppm Mo to produce the result.

CONTROL OF ZINC DEFICIENCY AND EXCESS

Zinc deficiency

Spray and soil applications, injections into tree trunks, and driving zinc-coated nails or pieces of galvanized iron into the trunks and limbs of trees have all been used as corrective treatments for zinc deficiency with varying degrees of success depending upon the crop, soil and climate.

In general, spray applications using zinc sulfate have been the most generally used and successful on trees. Used on foliage of fruit trees, it is necessary to add enough lime or soda ash ($^1/_2$ the weight of zinc sulfate) to safen the spray.

Zinc chelates applied to the soil are showing promise with some crops. For example, Boawn, Viets, and Crawford (1957) compared zinc uptake from soil in pots when zinc chelate, zinc sulfate, ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$, zinc frits and blast furnace slag, respectively, were added. Best recovery was from the chelate.

In the tabulation (Table II) which follows are given examples of corrective measures with various crops.

Zinc excess

Instances of zinc excesses under field conditions are not very common, though there are places where soils derived from or influenced by zinc-bearing ores or mining operations have to come to light, and zinc-rich peats have been uncovered in NewYork. It is certain that with the growing use of zinc salts for the control of zinc deficiency in a wide range of crops that zinc will build up in some soils to toxic concentrations.

There is need, as stated, for much more data, both soil and plant, to determine the threshold values beyond which injury from zinc is likely to occur.

The data at hand suggest that where excess zinc occurs application of lime-stone or phosphate will be helpful in lowering zinc solubility and absorption by plants. Millikan (1947b), working with controlled nutrient solutions, found that molybdenum salts decreased zinc toxicity. However, it is doubtful that practical use of this should be made, save in molybdenum-deficient areas, since excessive molybdenum absorption by forage is a threat to animal health.

TABLE II

Methods of correcting zinc deficiency and excess

ALFALFA
(*Medicago sativa*)

SPRAYS

A 0.5% $ZnSO_4 \cdot 7H_2O$ spray on young alfalfa effected marked recovery in 10 days (Boawn and Viets 1952).

ALMOND
(*Prunus amygdalus*)

SPRAYS

Dormant sprays of 15 to 25 lbs. zinc sulfate per 100 gal. water were found effective. In several cases, a summer foliar spray of 10 lbs. zinc sulfate, 10 lbs. lime hydrate to 100 gal. water may be needed. (Proebsting and Serr 1954).

APPLE
(*Malus sylvestris*)

SPRAYS

Dormant sprays of 25–50 lbs. zinc sulfate per 100 gal. water have been found effective (Boynton 1954).

AVOCADO
(*Persea americana*)

SOIL TREATMENT METHODS

Wallihan, Embleton and Printy (1958) found that zinc chelates (zinc ethylene diamine tetraacetate and zinc hydroxyethyl ethylene diamine triacetate) applied dry to soil or dissolved in irrigation water at rates of 1 pound per tree on 6-year-old trees raised zinc from 15 to 50 ppm in the leaves and maintained correction for at least $2^1/_2$ years.

Continued on next page

TABLE II (continued)

Methods of correcting zinc deficiency and excess

AVOCADO (continued)

SPRAYS

Foliar sprays containing 2 lbs. of zinc sulfate, 1 lb. of lime hydrate to 100 gal. water applied preferably in late winter or spring before the spring growth flush are effective (Lynch 1954).

BEAN

(Bountiful variety)

SOIL TREATMENT METHODS

Powers and Pang (1947) found that 40 to 80 lbs. zinc sulfate per acre on Powell silt loam (pH 5.5 to 6.5) produced good correction. Use of 320 lbs./acre was toxic to sunflowers and gooseberries.

SPRAYS

Viets (1951) applied a 0.3% $ZnSO_4 \cdot 7H_2O$ foliar spray to Red Mexican beans when they were 4–6" high and got excellent correction.

CHERRY

(*Prunus avium*, etc.)

SPRAYS

Dormant sprays of 50 lbs. zinc sulfate per 100 gal. water, supplemented by foliar spray of 12 lbs. zinc sulfate and 6 lbs. lime hydrate to 100 gal. water 30 days after leaves appear, were effective. (McWhorter 1937; McWhorter *et al.* 1939).

CITRUS

(Various species and varieties)

SOIL TREATMENT METHODS

Soil treatments have produced recovery, but sometimes severe injury. Because of the uncertainties attached to soil application of zinc sulfate, foliar spray applications are the preferred methods of control currently.

Leonard, Stewart and Edwards (1957) found that mixing 5 lbs. zinc sulfate and 5 lbs. calcium chloride and applying in small piles to sandy Florida soils greatly increased zinc uptake by the tree. The calcium apparently prevents zinc fixation by the soil and assures penetration into the root zone.

SPRAYS

Foliar sprays of 5 lbs. zinc sulfate, $2^1/_2$ lbs. lime hydrate to 100 gal. water applied before spring growth cycle or in early summer are effective. 10–5–100 formulations gave longer control. Various zinc dusts effective but zinc sulfate-lime sprays recommended by Parker (1938).

Zinc oxide at rates of $^1/_4$ lb. per 100 gal. can be included with oil sprays, the latter used for pest control.

Zinc sulfate at rates of 3 to 5 lbs. per 100 gal. safened with half as much lime hydrate can be used with urea and many other spray formulations. However, it should not be used with lime sulfur in the spring following blossom petal fall as it aggravates fruit scarring from the lime sulfur (Lewis 1946).

Johnston (1946) found that zinc dusts in the form of $ZnSO_4$, ZnO, ZnS, and metallic Zn diluted with talc were effective in mottle leaf control but that $ZnSO_4$ was best. Sprays were about twice as effective as dusts.

Steyn and Eve (1956) found that the incorporation of copper in zinc sprays decreased zinc absorption by the leaf.

Heymann-Herschberg (1956) found that a zinc-sulfur dust containing 90% sulfur and 10% ZnO (particle size of latter <0.15 mm) gave control of both rust mite and zinc deficiency. Lasting effect was only 6 months.

CORN

(*Zea mays*)

SOIL TREATMENT METHODS

Barnette, Camp, Warner and Gall (1936) corrected chlorosis (white bud) of corn by application of 10–20 lbs. of 89% zinc sulfate ($ZnSO_4 \cdot 7H_2O$) per acre on a Florida medium fine sand. The fertilizer was applied in the row. Stable manure was also helpful. Superphosphate applied with the zinc decreased its effectiveness.

Continued on next page

TABLE II (continued)

Methods of correcting zinc deficiency and excess

CORN (continued)

SPRAYS

Viets (1951) secured good correction on corn with a spray containing 0.5% $ZnSO_4 \cdot 7H_2O$;, and 0.25% $Ca(OH)_2$ sprayed on 6-inch-high corn. In later work, Viets *et al.* (1953) got no yield responses from sprays where there was moderate symptom expression and at leaf values (6th node from base at silking stage) of 15 ppm Zn. Statement is made that 15 ppm adequate for yields at 100 to 125 bushels corn per acre.

OTHER INFORMATION

Allowing volunteer weeds and grasses to grow one or two years between corn crops reduced severity of white bud in corn (Barnette, Camp, Warner and Gall 1936).

GRAPE
(*Vitis* spp.)

SPRAYS

Dormant sprays containing 4 to 8 lbs. zinc sulfate per 100 gal. water were effective (Clore 1951).

With spur-pruned varieties, daubing fresh pruning cuts in December with zinc sulfate sprays at a concentration of 1/2 lb. per gallon has been successful (Snyder and Harmon 1954).

OTHER INFORMATION

Foliar sprays have not been uniformly successful in correcting zinc deficiency of grapes; chelates have not shown advantages over inorganic sources in work to date (Cook 1958; and Cook and Mitchell 1958).

PEACH
(*Prunus persica*)

SOIL TREATMENT METHODS

Chandler (1937) corrected little leaf of peaches on sandy soils with 300 lbs. of zinc sulfate per acre applied in an area 2 feet from the tree trunks.

SPRAYS

Dormant sprays containing 15 lbs. zinc sulfate in 100 gal. water have proven successful.

Foliar sprays consisting of 10 lbs. zinc sulfate, 5 lbs. lime hydrate to 100 gal. water applied in spring and a dormant spray of from 10 to 50 lbs. zinc sulfate per gal. were found effective in South Africa by Dippenaar (1941).

Dormant spray applied in late winter of 50 lbs. zinc sulfate to 100 gal. water effective in Australia. (Baxter 1957).

OTHER INFORMATION

Zinc-coated shingle nails, 30 to 40 per tree with large, bearing peaches, have proven satisfatory. The heads of the nails should be removed (McWhorter 1945).

PEAR
(*Pyrus communis*)

SPRAYS

Dormant sprays of 25 lbs. zinc sulfate per 100 gal. water effective (Lindner and Luce 1944).

PECAN
(*Carya illinoensis*)

SOIL TREATMENT METHODS

Finch and Kinnison (1934) found that 10 lbs. zinc sulfate applied in a ring around trunks at a radius of about 2 feet gave control on alkaline soils. This rate was recommended for trees with a diameter of 10 inches. Lesser amounts are recommended for smaller trees.

SPRAYS

Spraying early spring growth with solution containing 4 lbs. zinc sulfate, 2 lbs. lime hydrate to 100 gal. water annually usually suffices. (Blackmon and Sharpe 1951).

Continued on next page

TABLE II (continued)

Methods of correcting zinc deficiency and excess

SUBTERRANEAN CLOVER

(*Trifolium subterraneum*)

SOIL TREATMENT METHODS

ZnO at 4 lbs. per acre or $ZnSO_4$ at $5^1/_2$ lbs. per acre on sandy soils gave control (Dunne and Elliott 1950).

SWEET CORN

(*Zea Mays*)

SPRAYS

Lingle and Holmberg (1957) found that a spray containing 0.5% $ZnSO_4 \cdot 7H_2O$ at the rate of 100 gal. per acre applied when the plants had 3 leaves and again when they had 5 leaves corrected the deficiency. Manganese sulfate added to the zinc increased zinc absorption.

TOBACCO

(*Nicotiana tabacum*)

SOIL TREATMENT METHODS

Bacon, Leighty and Bullock (1950) applied 1.2 and 5 lbs. Zn as $ZnSO_4$ per acre to to a Marlboro fine sandy loam of *p*H 5.5 and analysis of the tobacco leaves showed substantial uptake of zinc from all three rates. The average leaf content for 2 years was for check, 2, and 5 lbs. Zn, respectively, 26.4, 97.2, and 148.0 ppm in the dry matter.

TOMATO

(*Lycopersicon esculentum*)

SOIL TREATMENT METHODS

Lingle, Holmberg and Zobel (1958) found that tomatoes at setting out time watered with a solution containing 0.2% zinc sulfate and applied at 500 gal. per acre gave correction and increased the zinc content of the leaves.

TUNG

(*Aleurites* sp.)

SOIL TREATMENT METHODS

Mowry and Camp (1934) cured bronzing of tung trees on sandy soils by applying 0.25 to 0.50 lbs. zinc as zinc sulfate per tree.

WALNUT

(*Juglans regia*)

SPRAYS

Neither foliar nor dormant sprays are very effective (Proebsting and Serr 1954).

OTHER INFORMATION

Drive pieces of 22- to 24-gauge galvanized iron, 2 inches long and 7/8-inch wide, through bark and one-half inch into sapwood. Stagger in a pattern 2 inches apart in all directions at convenient points on major limbs. Drive parallel with grain of wood (Proebsting and Serr 1954).

MISCELLANEOUS

SOIL TREATMENT METHODS

Viets, Boawn and Crawford (1954) decreased the severity of zinc deficiency symptoms on castor beans, field beans, lima beans, sweet corn, field corn, sudan grass, Concord grapes, hops, soybeans, flax, and tomatoes when 23 lbs. Zn/acre as $ZnSO_4$ was applied to fine sandy loams of *p*H 8.2 to 8.5 at seeding stage.

REFERENCES

ALBEN, A. O. AND BOGGS, HUGH M., 1936, Zinc content of soils in relation to pecan rosette, *Soil Sci.*, **41**, 329–332.

ALBEN, A. O., COLE, J. R. AND LEWIS, R. D., 1932, New developments in treating pecan rosette with chemicals, *Phytopathology*, **22**, 979–981.

BACON, C. W., LEIGHTY, WILBUR R. AND BULLOCK, JAMES F., 1950, Boron, copper, manganese, and zinc requirement tests of tobacco, *U.S. Dept. Agr. Tech. Bull.* 1009, 1–27.

BARNETTE, R. M., 1936, The occurrence and behavior of less abundant elements in soils, *Florida Agr. Expt. Sta. Ann. Rept.* 1936, 61.

BARNETTE, R. M., CAMP, J. P., WARNER, J. D. AND GALL, O. E., 1936, The use of zinc sulphate under corn and other field crops, *Florida Agr. Expt. Sta. Bull.* 292, 1–51.

BARNETTE, R. M. AND WARNER, J. D., 1935, A response of chlorotic corn plants to the application of zinc sulfate to the soil, *Soil Sci.*, **39**, 145–156.

BATHURST, A. C., 1945, The effects of superphosphate on orange trees, *Farming in S. Africa*, **20**, 351–353.

BAXTER, P., 1957, Trace element deficiencies in fruit trees on Mallee and Wimmera soils, *Jour. Dept. Agr., Victoria*, **55**, 11–15, 17, 19–20, 26.

BERGH, H., 1947a, Zinc as a plant nutrient and plant poison, *Nord. JordbrForsk.* 1947, 121–130.

BERGH, H., 1947b, Plant-available zinc in soil. A comparison between chemically determined plant-available zinc and the amount of zinc found in plant tissues in growth experiments, *Kgl. Norske Videnskab. Selskabs, Forh.*, **20**, 41–44.

BERGH, H., 1948, Zinc as a plant nutrient and a plant poison. Water cultures and field experiments with grain, *Kgl. Norske Videnskab, Selskabs, Skrifter* 1942–45, No. 3, 66 pp.

BINGHAM, FRANK T. AND MARTIN, JAMES P., 1956, Effects of soil phosphorus on growth and minor element nutrition of citrus, *Proc. Soil Sci. Soc. Am.*, **20**, 382–385.

BLACKMON, G. H. AND SHARPE, R. H., 1951, Pecan growing in Florida, *Florida Agr, Expt. Sta. Bull.*, 437, 1–71.

BOAWN, L. C. AND VIETS, FRANK G., Jr., 1952, Zinc deficiency of alfalfa in Washinton. *Agron. Jour.*, **44**, 276.

BOAWN, LOUIS C., VIETS, FRANK G., Jr. AND CRAWFORD, CARL L., 1957, Plant utilization of zinc from various types of zinc compounds and fertilizer materials, *Soil Sci.*, **83**, 219–227.

BOLLARD, E. G., 1953, Zinc deficiency in pears, *New Zealand Jour. Sci. Technol.*, Sec. A**34**, 548–550.

BOULD, C., NICHOLAS, D. J. D., TOLHURST, J. A. H., WALLACE, T. AND POTTER, J. M. S., 1949, Zinc deficiency of fruit trees in Britain, *Nature*, **164**, 801–802.

BOULD, C., NICHOLAS, D. J. D., TOLHURST, J. A. H. AND POTTER, J. M. S., 1953a, Zinc deficiency of fruit trees in Great Britain, *Jour. Hort. Sci.*, **28**, 260–267.

BOULD, C., NICHOLAS, D. J. D. AND TOLHURST, J. A. H., 1953b, Copper and zinc deficiencies in soil and fruit trees in Britain, *Trans. Intern. Soc. Soil Sci. Comm. II and IV* 1952, **2**, 172–175.

BOYNTON, DAMON, 1954, Apple nutrition. Chapter I (pp. 1–78) in: *Mineral Nutrition of Fruit Crops*, Edited by N. F. Childers, Somerset Press, Somerville, New Jersey.

BRENCHLEY, WINIFRED E., 1927, *Inorganic Plant Poisons and Stimulants*, 2nd ed. 134 pp., Cambridge Univ. Press, London.

BRIGGS, LYMAN J., JENSEN, C. A. AND MCLANE, J. W., 1916, Mottle-leaf of citrus trees in relation to soil conditions, *Jour. Agr. Research*, **6**, 721–740.

CAMP, A. F., 1945, Zinc as a nutrient in plant growth, *Soil Sci.*, **60**, 157–164.

CAMP, A. F., CHAPMAN, H. D. AND PARKER, E. R., 1949, Symptoms of citrus malnutrition, Chapter 9 (pp. 307–365) in: *Hunger Signs in Crops*, Am. Soc. Agron. and National Fert. Assn., Washington, D.C.

CHANDLER, W. H., 1937, Zinc as a nutrient for plants, *Botan. Gaz.*, **98**, 625–646.

CHANDLER, W. H., HOAGLAND, D. R. AND HIBBARD, P. L., 1932, Little-leaf or rosette in fruit trees, *Proc. Am. Soc. Hort. Sci.*, **28** (1931), 556–560.

CHANDLER, W. H., HOAGLAND, D. R. AND HIBBARD, P. L., 1934, Little-leaf or rosette of fruit trees, III, *Proc. Am. Soc. Hort. Sci.*, **30** (1933), 70–86.

CHANDLER, W. H., HOAGLAND, D. R. AND MARTIN, J. C., 1946, Little-leaf or rosette of fruit trees, VIII: Zinc and copper deficiency in corral soils, *Proc. Am. Soc. Hort. Sci.*, **47**, 15–19.

Chapman, H. D., 1949, Citrus leaf analysis, *California Agr*, 3(11), 10, 12, 14.

Chapman, H. D., Liebig, George F. Jr. and Vanselow, A. P., 1939, Some nutritional relationships as revealed by a study of mineral deficiency and excess symptoms on citrus, *Proc. Soil Sci. Soc. Am.*, **4**, 196–200.

Chapman, H. D., Vanselow, A. P. and Liebig, George F. Jr., 1937, The production of citrus mottle-leaf in controlled nutrient cultures, *Jour. Agr. Research*, **55**, 365–379.

Childers, Norman F. (ed.) 1954, *Mineral Nutrition of Fruit Crops*, 907 pp., Somerset Press, Somerville, New Jersey.

Clore, W. J., 1951, Little leaf or zinc deficiency of Concord grapes, *Washington Agr. Expt. Sta. Circ.*, **136**, 3 pp. (Mimeographed.)

Conner, S. D., 1920, The effect of zinc in soil tests with zinc and galvanized iron pots, *Jour. Am. Soc. Agron.* **12**, 61–64.

Cook, James A., 1958, Field trials with foliar sprays of Zn-EDTA to control zinc deficiency in California vineyards, *Proc. Am. Soc. Hort. Sci.*, **72**, 158–164.

Cook, James, A. and Mitchell, Gordon F., 1958, Screening trials of chelated zinc materials toward the correction of zinc deficiency in Vinifera grapevine, *Proc. Am. Soc. Hort. Sci.*, **72**, 149–157.

Davidson, O. Wesley and Judkins, Wesley, P., 1949, Nutrient-deficiency symptoms in deciduous fruits. Chapter VII (pp. 215–267) in: *Hunger Signs in Crops*, Am. Soc. Agron. and National Fert. Assn., Washington, D.C.

Davies, Gwenda N., 1941, An investigation of the effect of zinc sulphate on plants, *Ann. Appl. Biol.*, **28**, 81–84.

Dippenaar, B. J., 1941, Diseases of fruit trees caused by leaf-rust, manganese, and zinc deficiencies, and their joint control, *S. African Jour. Sci.*, **37**, 136–155.

Drosdoff, Matthew, 1950, Minor-element content of leaves from tung orchards, *Soil Sci.*, **70**, 91–98.

Dunne, T. C. and Elliott, H. G., 1950, Zinc fertilizers for subterranean clover at Kendenup, *Jour. Dept. Agr., W. Australia*, **27**, 115–117.

Elgabaly, M. M., 1950, Mechanism of zinc fixation by colloidal clays and related minerals, *Soil Sci.*, **69**, 167–173.

Finch, A. H., 1936, Zinc and other mineral constituents in relation to the rosette disease of pecan trees, *Jour. Agr. Research*, **52**, 363–376.

Finch, A. H. and Kinnison, A. F., 1933, Pecan rosette: Soil, chemical, and physiological studies, *Arizona Agr. Expt. Sta. Tech. Bull.*, **47**, 407–442.

Finch, A. H. and Kinnison, A. F., 1934, Zinc treatment of pecan rosette, *Arizona Agr. Ext. Serv. Circ.*, **82**, 1–11.

Franco, Coaracy M. and Mendes, Helo Camargo, 1954, Deficiência de zinco em Cafeeiro (Zinc deficiency in the coffee bush), *Bol. Suptda. Serv. Café, S. Paulo*, **29** (334), 34–39.

Gaddum, L. W., Camp, A. F. and Reuther, Walter, 1936, Spectrographic studies of the composition of tissues and corresponding soils of normal and physiologically diseased horticultural crops, *Florida Agr. Expt. Sta. Rept. for* 1936, 82–84.

Gall, Owen E., 1936, Zinc sulphate studies in the soil, *Citrus Ind.* **17** (1), 20–21.

Gall, O. E. and Barnette, R. M., 1940, Toxic limits of replaceable zinc to corn and cowpeas grown on three Florida soils, *Jour. Am. Soc. Agron.*, **32**, 23–32.

Goodall, D. W. and Gregory, F. G., 1947, Chemical composition of plants as an index of their nutritional status, *Imperial Bur. Hort. and Plantation Crops, Tech. Commun.*, **17**, 167 pp.

Haas, A. R. C., 1936, Zinc relation in mottle-leaf of citrus, *Botan. Gaz.*, **98**, 65–86.

Hawkins, J. and Cameron, D. I., 1953, Effect of galvanized iron on some tree seedlings, *Nature*, **171**, 977–978.

Healy, W. B., 1952, Note on zinc deficiency of citrus at Aitutaki, Cook Islands, *New Zealand Jour. Sci. Technol.*, Sec. A34, 228–229.

Hewitt, Eric J., 1948, Relation of manganese and some other metals to the iron status of plants, *Nature*, **161**, 489–490.

Heymann-Herschberg, Lotte, 1956, Effects of combined zinc and sulfur applications on zinc deficiency in orange trees, *Ktavim*, **6**, 83–89.

Hibbard, P. L., 1940a, A soil zinc survey in California, *Soil Sci.*, **49**, 63–72.

Hibbard, P. L., 1940b, Accumulation of zinc on soil under long-persistent vegetation, *Soil Sci.*, **50,** 53–55.

Hoagland, D. R. and Arnon, D. I., 1938, The water-culture method for growing plants without soil, *California Agr. Expt. Sta. Circ.*, **347,** 1–39.

Hoagland, D. R., Chandler, W. H. and Hibbard, P. L., 1936, Little-leaf or rosette of fruit trees. V. Effect of zinc on the growth of plants of various types in controlled soil and water culture experiments, *Proc. Am. Soc. Hort. Sci.*, **33**(1935), 131–141.

Hoagland, D. R., Chandler, W. H. and Stout, P. R., 1937, Little-leaf or rosette of fruit trees. VI. Further experiments bearing on the cause of the disease, *Proc. Am. Soc. Hort. Sci.*, **34**(1936), 210–212.

Holmes, R. S., 1944, The effect of liming the soil upon the availability to plants of zinc and copper. *U.S. Dept. Agr., Bur. Plant Industry, Soils, & Agr. Engineering, Div. Soil & Fert. Investigations*, Research Report No. 23.

Hunter, James G. and Vergnano, Ornella, 1953, Trace-element toxicities in oat plants, *Ann. Appl. Biol.*, **40,** 761–777.

Javillier, M., 1908, Le zinc chez les plantes, *Ann. Inst. Pasteur*, **22,** 720–727.

Javillier, M., 1912, Sur l'emploi du zinc comme engrais catalytique, *8th Intern. Congr. App. Chem.*, **15,** 145–146.

Javillier, M., 1914a, Une cause d'erreur dans l'étude de l'action biologique des élémentes chimiques: la présence de traces de zinc dans le verre, *Acad. Sci. (Paris) Compt. Rend.*, **158,** 140–143.

Javillier, M., 1914b, Utilité du zinc pour la croissance de l'*Aspergillus niger* (*Sterigmatocystis nigra* V. Tgh.), *Acad. Sci. (Paris) Compt. Rend.*, **158,** 1216–1219.

Johnston, J. C., 1933, Zinc sulfate promising new treatment for mottle leaf, *California Citrograph*, **18,** 107, 116–118.

Johnston, J. C., 1946, Trials with zinc-bearing dusts, *California Citrograph*, **31,** 406–407.

Jones, H. A., Brown, B. E. and Houghland, G. V. C., 1949, Plant-nutrient deficiency symptoms in the potato. Chapter IV (pp. 107–134) in: *Hunger Signs in Crops*. Am. Soc. Agron. and National Fert. Assn., Washington, D. C.

Jurinak, J. J. and Thorne, D. W., 1955, Zinc solubility under alkaline conditions in a zinc-bentonite system, *Proc. Soil Sci. Soc. Am.*, **19,** 446–448.

Klosterman, H. J. and Clagett, C. O., 1956, Zinc content of potato leaves, *N. Dakota Agr. Expt. Sta. Bimonthly Bull.*, **19**(1), 24–27.

Labanauskas, C. K., Embleton, T. W. and Jones, W. W., 1958, Effects of phosphate on micronutrients in avocado leaves, *California Citrograph*, **43,** 133–134.

Leggett, G. E., 1952, M.S. Thesis, Utah State Agr. College, Logan, Utah.

Leonard, C. D., Stewart, Ivan and Edwards, George, 1957, Effectiveness of different zinc fertilizers on citrus, *Citrus Ind.*, **38**(2), 9, 10, 12–15.

Lewis, H. C., 1946, Spray injury from zinc–lime sulfur in Central California, *California Citrograph*, **31,** 112.

Lindner, R. C. and Luce, W. A., 1944, Zinc treatment for the control of rosette or little leaf of fruit trees, *Proc. Washington Hort. Soc.*, **40,** 154–160.

Lingle, John C. and Holmberg, D. M., 1957, The response of sweet corn to foliar and soil zinc applications on a zinc deficient soil, *Proc. Am. Soc. Hort. Sci.*, **70,** 308–315.

Lingle, John C., Holmberg, D. M. and Zobel, M. P., 1958, The correction of zinc deficiency of tomatoes in California, *Proc. Am. Soc. Hort. Sci.*, **72,** 397–402.

Loneragan, J. F., 1951, The effect of applied phosphate on the uptake of zinc by flax, *Australian Jour. Sci. Research*, Series **B4**, 108–114.

Lott, Wreal L., 1938, The relation of hydrogen-ion concentration to the availability of zinc in soil, *Proc. Soil Sci. Soc. Am.*, **3,** 115–121.

Lyman, Clarence and Dean, L. A., 1942, Zinc deficiency of pineapples in relation to soil and plant composition, *Soil Sci.*, **54,** 315–324.

Lynch, S. John, 1954, Avocado and mango, Chapter II (pp. 79–120) in: *Mineral Nutrition of Fruit Crops*, Edited by N. F. Childers, Somerset Press, Somerville, New Jersey.

Lyon, C. B., Beeson, K. C. and Ellis, G. H., 1943, Effects of micro-nutrient deficiencies on growth and vitamin content of the tomato, *Botan. Gaz.*, **104,** 495–514.

MASSEY, H. F., 1957, Relation between dithizone-extractable zinc in the soil and zinc uptake by corn plants, *Soil Sci.*, **83**, 123–129.

MAZE, P., 1914, Influences respectives des éléments de la solution minérale sur le développement du mais, *Ann. Inst. Pasteur*, **28**, 21–46.

MAZE, P., 1915, Détermination des éléments minéraux rares nécessaires au développement du mais, *Acad. Sci (Paris) Compt. Rend.*, **160**, 211–214.

MCCLUNG, A. C., 1954, The occurrence and correction of zinc deficiency in North Carolina peach orchards, *Proc. Am. Soc. Hort. Sci.*, **64**, 75–80.

MCGEORGE, W. T., 1939, Some problems connected with fertilization of alkali soils, *California Citrograph*, **24**, 389, 424.

MCGEORGE, W. T., 1949, A study of lime-induced chlorosis in Arizona orchards, *Ariz. Agr. Expt. Sta. Tech. Bull.*, **117**, 341–388.

MCMURTREY, J. E., Jr., 1948, Visual symptoms of malnutrition in plants. Chapter 8 (pp. 231–289) in *Diagnostic Techniques for Soils and Crops*, Am. Potash Institute, Washington 6, D.C.

MCMURTREY, J. E., Jr., 1949, Plant-nutrient deficiency in tobacco. Chapter 11 (pp. 19–58), in: *Hunger Signs in Crops*, Am. Soc. Agron. and National Fert. Assn., Washington, D. C.

MCWHORTER, O. T., 1937, Some observations of horticultural practices, *Oregon State Hort. Soc. Ann. Rept.*, **29**, 68–73.

MCWHORTER, O. T., 1945, Zinc-coated nails check "little-leaf," *Better Fruit*, **39** (10), 11.

MCWHORTER, O. T., LAWRENCE, W. W. AND LEAR, GENE, 1939, Little leaf control demonstration *Oregon State Hort. Soc. Ann. Rept.*, **31**, 110–115.

MILLIKAN, C. R., 1942, Symptoms of zinc deficiency in wheat and flax, *Jour. Australian Inst. Agr. Sci.*, **8**, 33–35.

MILLIKAN, C. R., 1947a, Effect of phosphates on the development of zinc deficiency symptoms in flax, *Jour. Dept. Agr., Victoria*, **45**, 273–278.

MILLIKAN, C. R., 1947b, Effect of molybdenum on the severity of toxicity symptoms in flax induced by an excess of either manganese, zinc, copper, nickel, or cobalt in the nutrient solution, *Jour. Australian Inst. Agr, Sci.*, **13**, 180–186.

MILLIKAN, C. R., 1953, Relative effects of zinc and copper deficiencies on lucerne and subterranean clover, *Australian Jour. Biol. Sci.*, **6**, 164–177.

MOWRY, HAROLD AND CAMP, A. F., 1934, A preliminary report on zinc sulphate as a corrective for bronzing of tung trees, *Florida Agr. Expt. Sta. Bull.*, **273**, 1–34.

NELSON, WERNER L. AND BEAR, FIRMAN E., 1949, Plant-nutrient deficiency symptoms in legumes. Chapter VIII (pp. 269–305) in: *Hunger signs in Crops*, Am. Soc. Agron. and National Fert. Assn., Washington, D. C.

OZANNE, P. G., 1955, The effect of nitrogen on zinc deficiency in subterranean clover, *Australian Jour. Biol. Sci.*, **8**, 47–55.

PARKER, E. R., 1934, Experiments on the treatment of mottle-leaf of citrus trees, *Proc. Am. Soc. Hort. Sci.*, **31** (1934), 98–107.

PARKER, E. R., 1938, Experiments on the treatment of mottle-leaf of citrus trees, IV. *Proc. Am. Soc. Hort. Sci.*, **35** (1937), 217–226.

PIPER, C. S., 1940, The symptoms and diagnosis of minor-element deficiencies in agricultural and horticultural crops. Pt. II. Copper, zinc, molybdenum, *Empire Jour. Expt. Agr.*, **8**, 199–206.

POWERS, W. L. AND PANG, T. S., 1947, Status of zinc in relation to Oregon soil fertility, *Soil Sci.*, **64**, 29–36.

PROEBSTING, E. L. AND SERR, E. F., 1954, Edible nuts. Chapter 9 (pp. 477–494) in: *Mineral Nutrition of Fruit Crops*, Edited by N. F. Childers, Somerset Press, Somerville, New Jersey.

RAULIN, J., 1863, Etudes chimiques sur la végétation des Mucédinées, particuliérement de l'*Ascophora nigrans*, *Acad. Sci. (Paris) Compt. Rend.*, **57**, 228–230.

RAULIN, J., 1869, Etudes chimiques sur la végétation, *Ann. Sci. Nat.*, **11**, 93–299.

RAULIN, J., 1870, Sur les conditions chimiques de la vie des organismes inférieurs, *Acad. Sci. (Paris) Compt. Rend.*, **70**, 634–638.

REUTHER, WALTER AND CRAWFORD, C. L., 1946, Effect of certain soil and irrigation treatments on citrus chlorosis in a calcareous soil: 1. Plant responses, *Soil Sci.*, **62**, 477–491.

REUTHER, WALTER AND SMITH, PAUL F., 1950, A preliminary report on the relation of nitrogen, potassium, and magnesium fertilization to yield, leaf composition, and the incidence of zinc deficiency in oranges, *Proc. Am. Soc. Hort. Sci.*, **56**, 27–33.

REUTHER, WALTER AND SMITH, PAUL F., 1954, Leaf analysis of citrus. Chapter 7 (pp. 257–294) in: *Mineral Nutrition of Fruit Crops*, Edited by N. F. Childers, Somerset Press, Somerville, New Jersey.

ROBINSON, W. O., LAKIN, H. W. AND REICHEN, LAURA E., 1947, The zinc content of plants on the Friedensville zinc slime ponds in relation to biogeochemical prospecting, *Econ. Geol.*, **42**, 572–582.

ROGERS, LEWIS H. AND WU, CHIH-HWA, 1948, Zinc uptake by oats as influenced by application of lime and phosphate, *Jour. Am. Soc. Agron.*, **40**, 563–566.

SHAW, ELLSWORTH AND DEAN, L. A., 1952, Use of dithizone as an extractant to estimate the zinc nutrient status of soils, *Soil Sci.*, **73**, 341–347.

SHEAR, CORNELIUS B., 1958, Responses of tung trees in sand and soil cultures to ratios of ammonium, to nitrate nitrogen and levels of potassium and zinc, *Proc. Am. Soc. Hort. Sci.*, **72**, 224–239.

SKINNER, J. J. AND DEMAREE, J. B., 1926, Relation of soil conditions and orchard management to the rosette of pecan trees, *U.S. Dept. Agr. Bull.*, 1378, 1–16.

SMITH, PAUL F. AND SPECHT, ALSTON W., 1953, Heavy-metal nutrition and iron chlorosis of citrus seedlings, *Plant Physiol.*, **28**, 371–382.

SNYDER, ELMER AND HARMON, FRANK N., 1954, Some responses of Vinifera grapes to zinc sulphate, *Proc. Am. Soc. Hort. Sci.*, **63**, 91–94.

SOMMER, A. L., 1927, The search for elements essential in only small amounts for plant growth, *Science*, **66**, 482–484.

SOMMER, A. L., 1928, Further evidence of the essential nature of zinc for the growth of higher green plants, *Plant Physiol.*, **3**, 217–221.

SOMMER, A. L., AND LIPMAN, C. B., 1926, Evidence on the indispensable nature of zinc and boron for higher green plants, *Plant Physiol.*, **1**, 231–249.

STAKER, E. V., 1942, Progress report on the control of zinc toxicity in peat soils, *Proc. Soil Sci. Soc. Am.*, **7**, 387–392.

STAKER, E. V. AND CUMMINGS, R. W., 1941, The influence of zinc on the productivity of certain New York peat soils, *Proc. Soil Sci. Soc. Am.*, **6**, 207–214.

STEINBERG, R. A., 1918, A study of some factors influencing the stimulative action of zinc sulphate on the growth of *Aspergillus niger*. I. The effect of the presence of zinc in the cultural flasks, *Torrey Bot. Club Mem.*, **17**, 287–293.

STEINBERG, R. A., 1919, A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*, *Am. Jour. Botany*, **6**, 330–372.

STEYN, W. J. A. AND EVE, D. J., 1956, The zinc status of citrus and pineapples in the Eastern Cape, *S. African Journ. Sci.*, **52**, 270–271.

SWAINE, D. J., 1955, The trace-element content of soils, *Commonwealth Bur. Soil Sci. Tech. Commun.* No. 48. Commonwealth Agr. Bur., England.

TEAKLE, L. J. H. AND TURTON, A. G., 1943, The copper, manganese, and zinc content of subterranean clover and oats in Western Australia, *Jour. Dept. Agr., W. Australia*, **20**, 238–259.

THOMAS, E. E. and LAURANCE, B. L., 1933, The zinc content of mottled and green orange leaves. Unpublished data on file in the Dept. of Soils & Plant Nutrition, Univ. California Citrus Expt. Sta.

THOMAS, WALTER, MACK, WARREN B., SMITH, Cyril B. AND FAGAN, FRANK N., 1949, Foliar diagnosis: The range in the zinc content of young apple trees, *Proc. Am. Soc, Hort. Sci.*, **53**, 6–10.

THORNE, D. W., LAWS, W. DERBY AND WALLACE, ARTHUR, 1942, Zinc relationships of some Utah soils, *Soil Sci.*, **54**, 463–468.

THORNE, D. W. AND WANN, F. B., 1950, Nutrient deficiencies in Utah orchards, *Utah Agr. Expt. Sta. Bull.*, **338**, 1–29.

THORNE, WYNNE, 1957, Zinc deficiency and its control, *Advances in Agronomy*, **9**, 31–65.

TUCKER, T. C., KURTZ, L. T. AND LYNCH, D. L., 1953, Zinc status of some Illinois soils as estimated by an *Aspergillus niger* method, *Proc. Soil Sci. Soc. Am.*, **17**, 111–114.

TUCKER, T. C. AND KURTZ, L. T., 1955, A comparison of several chemical methods with the bioassay procedure for extracting zinc from soils, *Proc. Soil Sci. Soc. Am,*, **19**, 477–481.

VAN SCHREVEN, D. A., 1937, Zink als een noodzakelijk element voor de suikerbieten de aardappelplant. (Zinc as an essential element for sugar beet and potatoes.). *Meded. Inst. Suikerbiet.*, **7**, 1–26.

Viets, Frank G., Jr., 1951, Zinc deficiency of corn and beans on newly irrigated soils in Central Washington, *Agron. Jour.*, **43**, 150–151.

Viets, F. G., Jr., Boawn, L. C., Crawford, C. L. and Nelson, C. E., 1953, Zinc deficiency in corn in Central Washington, *Agron., Jour.*, **45**, 559–565.

Viets, F. G., Jr., Boawn, L. C. and Crawford, C. L., 1954, Zinc contents and deficiency symptoms of 26 crops grown on a zinc-deficient soil, *Soil Sci.*, **78**, 305–316.

Viets, F. G., Jr., Boawn, L. C. and Crawford, C. L., 1957, The effect of nitrogen and types of nitrogen carrier on plant uptake of indigenous and applied zinc, *Proc. Soil Sci. Soc. Am.*, **21**, 197–201.

Wallace, T., 1953, *The Diagnosis of Mineral Deficiencies in Plants*, Chemical Publ. Co., Inc., New York.

Wallihan, E. F., Embleton, T. W. and Printy, Wilma, 1958, Zinc deficiency in the avocado, *California Agr.*, **12**(6), 4–5.

Walsh, T. and Clarke, E. J., 1945, Iron deficiency in tomato plants grown in an acid peat medium, *Proc. Royal Irish Acad.*, **50*B***, 359–372.

Wear, John I., 1956, Effect of soil *p*H and calcium on uptake of zinc by plants, *Soil Sci.* **81**, 311–315.

Wear, John I. and Sommer, Anna L., 1947, Acid-extractable zinc of soils in relation to the occurrence of zinc-deficiency symptoms of corn: A method of analysis, *Proc. Soil Sci. Soc. Am.*, **12**, 143–144.

West, E. S., 1938, Zinc-cured mottle leaf in citrus induced by excess phosphate, *Australia Council Sci and Indus. Res. Jour.*, **11**, 182–184.

Winters, Eric and Parks, W. L., 1955, Zinc deficiency of corn, *Tennessee Farm & Home Sci. Progress Rept.*, **10** (i.e., 14), 6, 8, Apr./June.

Wood, J. G. and Sibly, Pamela M., 1950, The distribution of zinc in oat plants, *Australian Jour. Sci. Research, Ser. B3*, 14–27.

Woodbridge, C. G., 1951, A note on the incidence of zinc deficiency in the Okanagan Valley of British Columbia, *Sci. Agr.*, **31**, 40.

FUMURES MINERALES DE L'ARACHIDE AU SENEGAL

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RESUME

Les recherches de l'I.R.H.O. sur la fumure minérale de l'arachide ont commencé en 1951.

La réalisation de nombreuses expériences sur les points d'essais de Louga et Tivaouane (Nord Sénégal) au secteur I.R.H.O. du Centre de Recherches Agronomiques de Bambey (Centre Sénégal) au point d'essais de Darou (Sud Sénégal) ainsi qu'environ 120 essais factoriels (3^3 et $4 \times 4 \times 2$) réalisés par les assistants européens en diverses situations en dehors des stations, ont permis de déterminer 4 types de fumures minérales adaptés aux diverses régions du Sénégal.

Le diagnostic foliaire a rendu possible une interprétation rationnelle des expériences de fumures et sur la base des "niveaux critiques", une extrapolation des résultats expérimentaux. On a pu tracer ainsi une sorte de carte de la nutrition minérale de l'arachide au Sénégal.

Pour les faibles doses d'engrais employés (entre 100 et 150 kg à l'hectare), le placement en side-dressing est supérieur à l'épandage à la volée. Un distributeur d'engrais, pouvant être accouplé au semoir, a été mis au point.

La comparaison de divers engrais phosphorés (phosphates bicalciques et tricalciques, superphosphate simple, phospal), à montré que, en fumure annuelle et pour de faibles doses, l'utilisation du phosphate tricalcique est à proscrire. Les résultats les plus constants ont été obtenus avec le phosphate bicalcique. Le phospal peut étre utilisé lorsque la pluviosité dépasse 800 mm. En fumure de fond et à fortes doses, le phosphate tricalcique ("schlamms") donne des résultats intéressants. Des recherches en cours étudient l'augmentation de l'assimilabilité du phosphate tricalcique par acidification au moyen de soufre.

Dans de nombreuses situations, l'action du sulfate d'ammoniaque doit etre attribuée au soufre. L'application de divers composés soufrés ou de soufre élémentaire a donné des résultats positifs.

La valeur de ces résultats a été confirmée par des essais de démonstration en culture indigène. Dans la région de Kaolack, au sud du Sénégal, plus de 200 comparaisons entre fumure et pas de fumure ont montré une action *moyenne* de l'engrais de plus de 500 kg de gousses à l'hectare (Cet engrais est composé de 40 kg de sulfate d'ammoniaque, 60 kg de phosphate bicalcique et 20 kg de chlorure de potasse à l'hectare).

Une augmentation de la productivité de 50% est ainsi obtenue.

INTRODUCTION

Le Sénégal est un des principaux pays producteurs d'arachides (700,000 tonnes en moyenne pour les 3 dernières années).

Il présente la particularité d'avoir une zone de culture extrêmement concentrée ce qui justifie les efforts de vulgarisation actuellement poursuivis et permet à la Recherche d'avoir le maximum d'efficacité. Cette zone bien que peu étendue, possède des caractéristiques climatiques extrêmement variées. C'est pourquoi l'Institut de Recherche

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pour les Huiles et Oléagineux a préféré implanter de petites Stations bien représentatives dans différentes régions du Territoire, plutôt que de se concentrer sur une Station principale.

Les quatre points sur lesquels l'expérimentation a été poursuivie sont figurés sur la carte ci-jointe (Figure 1). Le Tableau I donne quelques caractéristiques écologiques de chacun d'eux.

Les sols sont pour la plupart extrêmement sableux et très pauvres. Ils s'enrichissent au fur et à mesure que l'on descend vers le Sud.

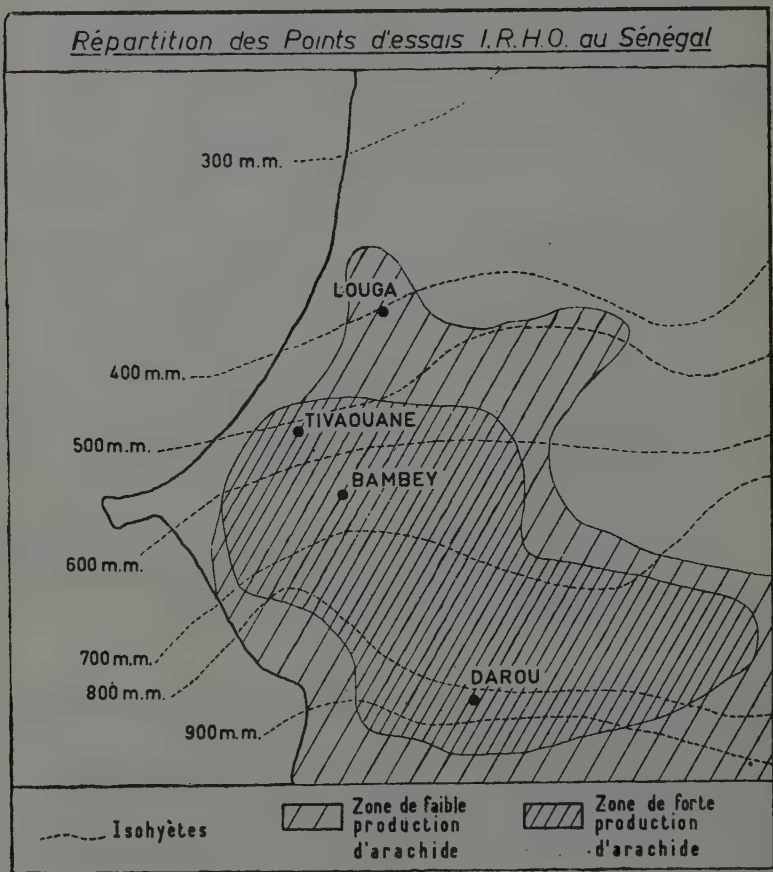


Figure 1

TABLEAU I

	Quantité d'eau (mm)	Teneur du sol	
		en argile (%)	en matière organique(‰)
Louga	415	1— 1.8	0.3— 1
Tivaouane	590	2— 2.5	2 — 3
Bambey	670	2— 3.5	2 — 6
Darou	850	3—15	5 —13

Les programmes des Stations sont surtout axés sur le problème de la nutrition minérale et en particulier sur l'établissement d'une carte des besoins en engrais. Dans cette intention sur chacun d'eux sont implantés des essais permanents de fumure (N – P – K – Ca – Mg ou oligo-éléments – formes d'engrais, etc.) suivis par le diagnostic foliaire. Ces essais en général du type factoriel permettent d'apprécier avec une grande précision les caractéristiques de la nutrition sur la Station et son évolution. A partir de ces données de base, par une série de petits essais à 27 ou 32 parcelles (3^3 ou $4 \times 4 \times 2$), toujours contrôlés par le diagnostic foliaire mais réalisés chez des cultivateurs Africains, on détermine quelle est l'importance de la région qui correspond aux mêmes caractéristiques de nutrition.

Ceci permet de délimiter alors de larges zones où certains éléments sont plus ou moins déficients et où par conséquent leur application permet d'obtenir de fortes augmentations de rendements. Lorsque la zone à déterminer est caractérisée par une carence ou un excès très accusé, on procède directement à des diagnostics foliaires en champs sans passer par l'intermédiaire des essais agronomiques.

A l'heure actuelle, un réseau d'environ 120 essais a couvert entièrement la zone arachidière et permis de tracer avec précision une carte des fumures minérales.

DIAGNOSTIC FOLIAIRE ET FUMURE

Généralités

L'étude de la fumure minérale par la seule utilisation des données classiques des essais agronomiques, ne permet pas d'expliquer certains résultats; le contrôle systématique de l'essais par les analyses foliaires de chaque parcelle fait mieux comprendre l'action des fumures grâce à l'examen des corrélations des éléments chimiques entre eux et avec les rendements. La valeur des essais ainsi conduits est fortement accrue puisque le diagnostic foliaire permet de sortir du cadre de l'empirisme de l'expérience pour voir et aussi, par les prélèvements en cultures africaines, d'extrapoler les résultats expérimentaux.

La réalisation pratique du diagnostic foliaire demande un certain nombre de précautions et de techniques que nous rappellerons rapidement. Les feuilles sont prélevées sur la tige principale des plantes (rang 4 à 6 en comptant à partir du bas) entre le 40ème et le 45ème jour après le semis.

Quand on opère sur un essai agronomique, on prélève sur les lignes qui seront récoltées et pesées en fin de végétation, très exactement 50 feuilles saines (exemptes de cercosporiose) et normales par parcelle.

Lorsqu'on cartographie une région pour déterminer les zones de carence ou d'excès (cas du phosphore par exemple), on exécute les prélèvements sur des cultures indigènes. On procède alors en 2 temps:

a) après une prospection systématique de la région à cartographier, quelques jours après le semis, on repère des champs dont les précédents culturaux sont semblables, qui ont été semés à la même date, et qui n'ont reçu aucun apport d'engrais.

b) 45 jours après le semis sur les champs repérés précédemment, on exécute le prélèvement en vérifiant qu'entre temps aucun traitement n'est venu perturber les conditions moyennes de culture. On récolte également 50 feuilles très exactement.

Ces feuilles sont lavées à l'eau distillée puis séchées au four à Infra-rouge à 100–105° et mises en sacs polyéthylènes soudés à chaud. Envoyés par avion au Siège de l'I.R.H.O. à Paris, les échantillons sont pesés (poids sec) et broyés.

L'azote est dosé par la méthode de Kjeldahl. Les autres dosages sont réalisés sur cendres. Le phosphore par photocolorimétrie (Vanado-molybdate). La potasse – le calcium – et le magnesium au Spectrophotomètre de flamme (flamme chaude, hydrogène, oxygène et atomiseur à très faible débit).

La centralisation des résultats est opérée sur fiches perforées et l'interprétation statistique, tant des analyses chimiques que des données agronomiques, est réalisée par machine I. B. M.

Exploitation des Résultats et Utilisation du Diagnostic Foliaire

L'exploitation des milliers d'analyses exécutées sur arachides a permis d'établir des normes de nutrition pour chaque élément. On a convenu de considérer ces normes comme des niveaux critiques, c'est-à-dire des niveaux de nutrition au delà desquels on n'obtient plus de réponse aux applications d'azote, phosphore, potasse, etc. Ces niveaux critiques ont été fixés théoriquement à:

3.5 pour l'azote
0.225 pour le phosphore
0.8–1.0 pour la potasse
1.2 pour le calcium

L'utilisation pratique de ces normes appelle quelques remarques.

Azote

Le niveau critique de l'azote fixé à 3.5 est en fait relié au poids sec des feuilles. On a vu précédemment que lors de l'exécution du prélèvement de diagnostic foliaire, on prenait soin de constituer uniformément des échantillons de 50 feuilles dont on faisait le poids sec. Ces données ont été utilisées pour l'établissement d'un graphique (Figure 2),

qui permet de savoir si la nutrition azotée est excédentaire, normale ou déficiente. On voit ainsi qu'une teneur de 3.55 est suffisante si le poids sec est compris entre 2.5 gr et 6 gr (Produit N x poids sec compris entre 9 et 21 gr).

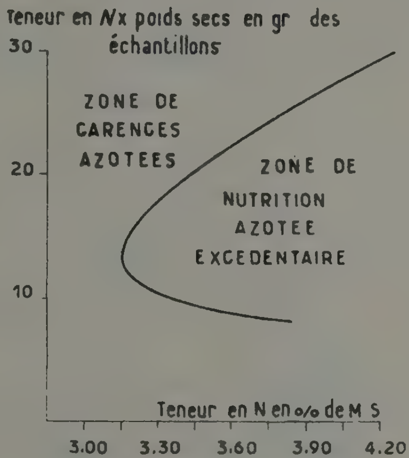


Figure 2
Courbe de référence pour la nutrition azotée

Il arrive cependant que l'on observe parfois des réponses pour des teneurs supérieures au niveau critique. En général, ces réponses sont obtenues avec des engrais azotés qui contiennent du soufre (cas du sulfate d'ammoniaque) et sont dues au soufre; un apport de sulfate de soude ou même de soufre en fleur permet d'obtenir les même résultats.

Phosphore

Les teneurs optima en phosphore varient selon les teneurs en azote. Une étude portant sur des milliers d'analyses a permit de tracer une courbe de réponse avec beaucoup de précision (Figure 3). A l'examen on voit que pour une teneur en azote de 3%, le niveau optimum en phosphore est de 0.200, pour 3.5 de 0.225, pour 4 de 0.250 etc.

Potasse

En ce qui concerne la potasse, le prélèvement de l'échantillon a une importance extrême. De nombreuses expériences conduites ces dernières années ont montré que le rang de la feuille prélevée avait une influence très sensible (en partie d'ailleurs en raison des variations de poids sec que cela entraînait) sur les teneurs en potasse et bien qu'une relation parabolique teneur en K – poids sec ait été mise en évidence, elle n'est valable que pour un rang donné.

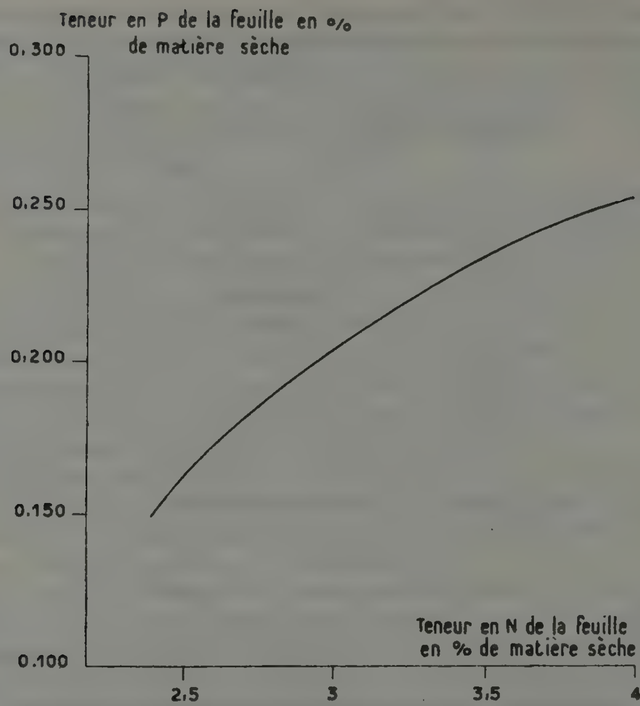


Figure 3
Courbe de référence pour la nutrition phosphorée.

L'étude de ces relations a été entreprise pour toute une série d'expériences et se trouve résumée dans la Figure 4. Les flèches indiquent l'évolution des teneurs en K et du poids sec sous l'action d'une fumure potassique, les signes '+' ou '—' indiquent si la réponse au rendement a été négative ou positive.

On peut dire en gros qu'une teneur de 0.6% en K correspond à une déficience pour des feuilles de rang 4 à 6 et que pour des feuilles de rang 8 cette déficience est certaine en-dessous de 0.8%.

Calcium — Magnesium

Au Sénégal, nous n'avons jamais observé de réponses importantes au calcium et au magnesium. L'ordre de grandeur des chiffres rencontrés permet de fixer comme niveau satisfaisant:

1.2–1.5% pour le calcium
0.5% pour le magnesium

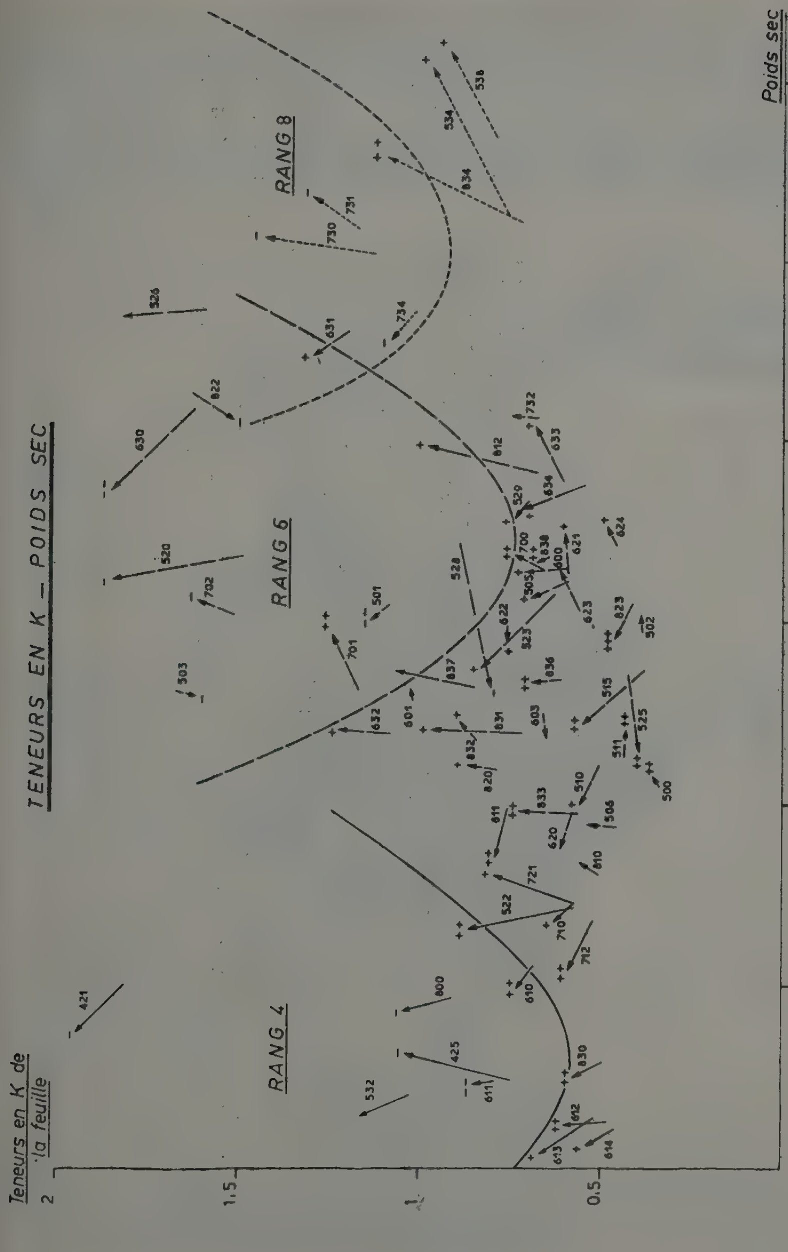


Figure 4
Courbes de référence pour la nutrition potassique.

Cartographie des Besoins en Engrais

L'ensemble des données acquises depuis la mise en route de nos essais permet de tracer maintenant avec précision une carte de fumure minérale (Figure 5). On s'est attaché à définir avec précision l'importance de certaines zones et pour cela nos essais scientifiques ont été complétés par des prospections systématiques de diagnostio foliaire en champs Indigènes (en particulier pour les zones à nutrition phosphorée excédentaire).

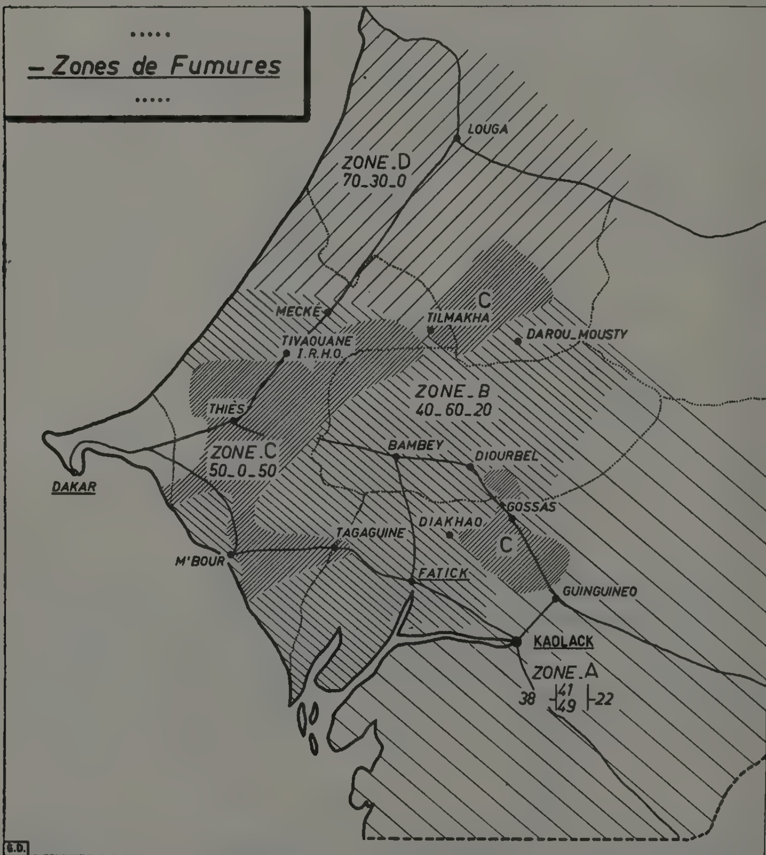


Figure 5

Carte des fumures minérales de l'arachide au Sénégal. Les doses pour chaque zone sont données en kg par hectare de sulfate d'ammoniaque, phosphate bicalcique (et phospal pour la zone A) et chlorure de potasse.

On distingue actuellement *quatre grandes zones de nutrition* pour lesquelles des fumures différentes et bien équilibrées sont seules susceptibles de fournir des augmentations de rendement certaines et constantes.

Zones A et B

Au Sud et au Centre du Territoire, on se trouve en présence d'une région assez homogène où les réponses aux engrais sont surtout importantes pour le *phosphore* (Tableau II).

TABLEAU II

Situation	Zone A	Zone B	
Détail	27 essais factoriels dans la région de Kaolack et sur la route de Tambacounda	10 essais factoriels aux environs de Tivaouane	23 essais factoriels dans le Baol et aux environs de Fatick
Effet de N*	+356 kg/ha	+137 kg/ha	+211 kg/ha
Effet de P	+514 "	+317 "	+258 "
Effet de K	+110 "	+ 65 "	+ 80 "
Augmentation totale à l'hectare	+880 "	+519 "	+549 "

* N = sulfate d'ammoniaque.

Les effets du sulfate d'ammoniaque et de la potasse sont moindres: cette dernière agit surtout quand on fertilise des sols qui ont supporté des cultures successives d'arachides.

L'utilisation des phosphates locaux a conduit à préconiser 2 formules suivant que l'on se trouvait dans les zones à forte ou à faible pluviosité.

En zone A. La formule actuellement vulgarisée convient parfaitement (5.5 – 22.2 – 8.8) et comprend environ:

38 kg de sulfate d'ammoniaque
41 kg de phosphate bicalcique
49 kg de phospal**
22 kg de chlorure de potassium

Une moitié du phosphore est appliquée sous forme de bicalcique, l'autre sous forme de phospal.

En zone B. L'utilisation d'une formule comprenant:

40 kg de sulfate d'ammoniaque
60 kg de phosphate bicalcique
20 kg de chlorure de potassium

assurera un maximum de rentabilité et un effet régulier.

** Phospal = phosphate d'alumine calciné. L'aluminium n'est toxique pour l'arachide qu'à de fortes concentrations (Sag 1956); 34% de P_2O_5 total.

Zone C

Au Nord des zones A et B, selon un tracé assez complexe qui est précisé sur une carte à plus grande échelle (carte des diagnostics foliaires en champs Indigènes — Figure 6), on rencontre une zone C dite '*Tache de Thiés*' qui prospectée systématiquement depuis 1954, s'est avérée assez étendue. Elle couvre en effet une grande partie du Cercle de Thiés.

On retrouve dans le Centre du Territoire, un ilot comparable (région de Gossas) dont l'existence avait été décelée pour la première fois par l'I.R.H.O. en 1956. Enfin, vers le Nord-Est la zone se prolonge au delà de Tilmakha.

La nutrition de cette région étant caractérisée par un niveau très élevé en phosphore, l'application d'une fumure phosphatée est généralement dépressive. L'expérience a montré que, même après plusieurs cultures successives avec un engrais azotopotassique, le besoin en phosphore était nul quand on avait au départ des teneurs du sol en P_2O_5 supérieures à 140 ppm (Ollagnier et Prevot 1956).

La réponse aux engrais, sur les 14 essais mis en place dans cette zone, est donc uniquement assurée par le *sulfate d'ammoniaque et la potasse*.

Sans phosphore, elle atteint 400 kg de gousses à l'hectare (Tableau III).

On limitera dans cette région, où la rosette (maladie à virus) sévit fréquemment, l'application d'engrais à 100 kg/ha afin de réduire les pertes que pourrait subir le cultivateur si sa récolte était atteinte par la maladie. On utilisera une formule comprenant:

50 kg de sulfate d'ammoniaque
50 kg de chlorure de potassium

Zone D

Elle se situe dans la partie Nord du Territoire de part et d'autre de la route Saint-Louis-Mecke. Les réponses aux différents engrais appliqués ne sont pas très fortes, mais cependant restent remarquablement constantes:

Seuls, *l'azote et le phosphore* procurent des augmentations de rendements. La potasse, dans cinq cas seulement sur vingt a un effet positif très faible, elle est parfois dépressive. Son action, en moyenne, peut être considérée comme nulle.

Les réponses aux engrais obtenues sur les vingt essais réalisés dans cette région sont données dans le Tableau IV.

Ceci confirme le observations faites précédemment (Prevot, Ollagnier et Fourrier 1953) dans la région de Louga. On se trouve en présence d'une carence azotée et phosphorée, cette dernière n'apparaissant souvent qu'une fois les besoins en azote satisfaits.

L'augmentation des rendements (+318 kg/ha) étant en moyenne plus faible que dans le Sud, il convient de ne pas employer une fumure trop onéreuse. Une dose de 100 kg/ha du mélange suivant semble suffisante:

70 kg de sulfate d'ammoniaque
30 kg de phosphate bicalcique

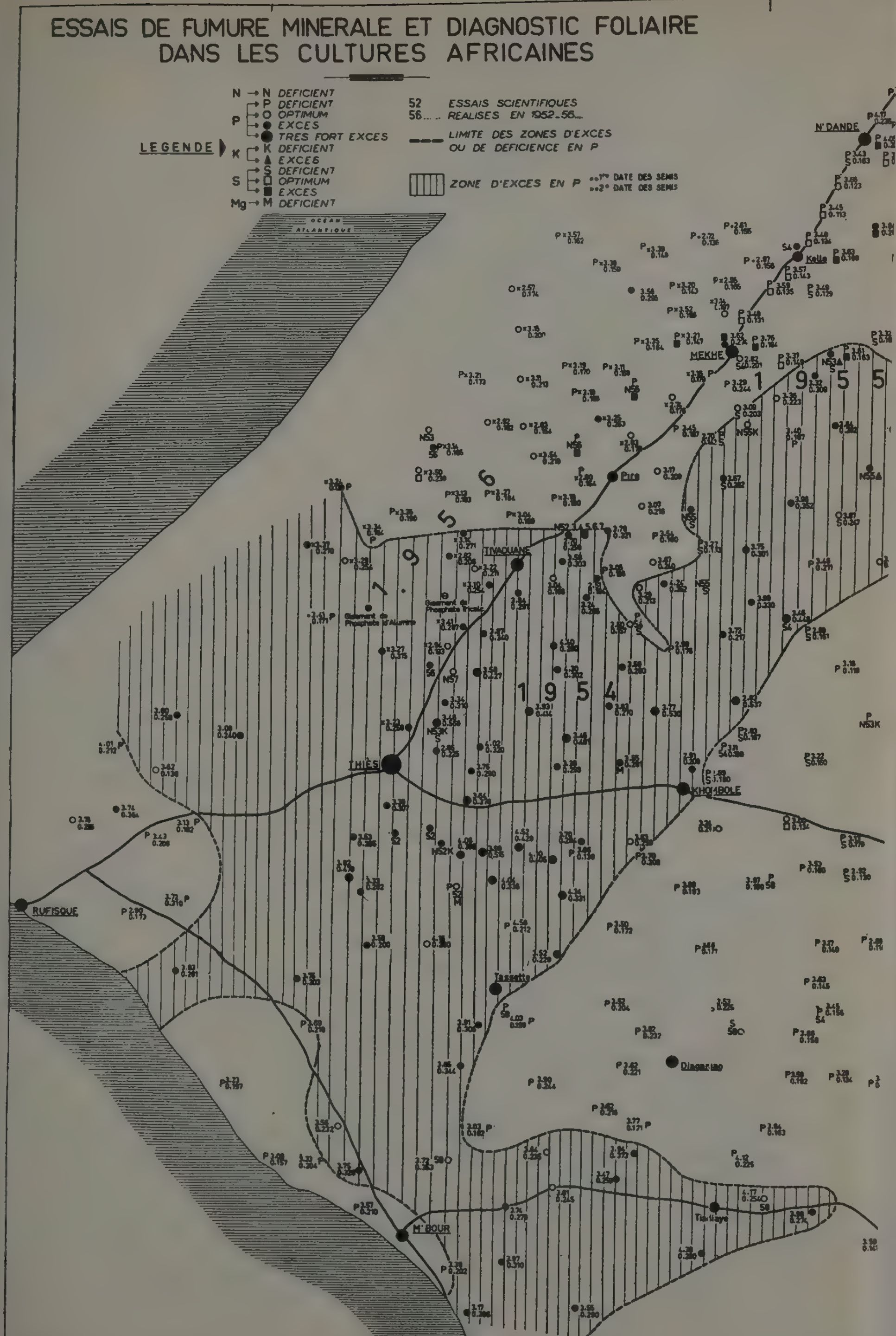
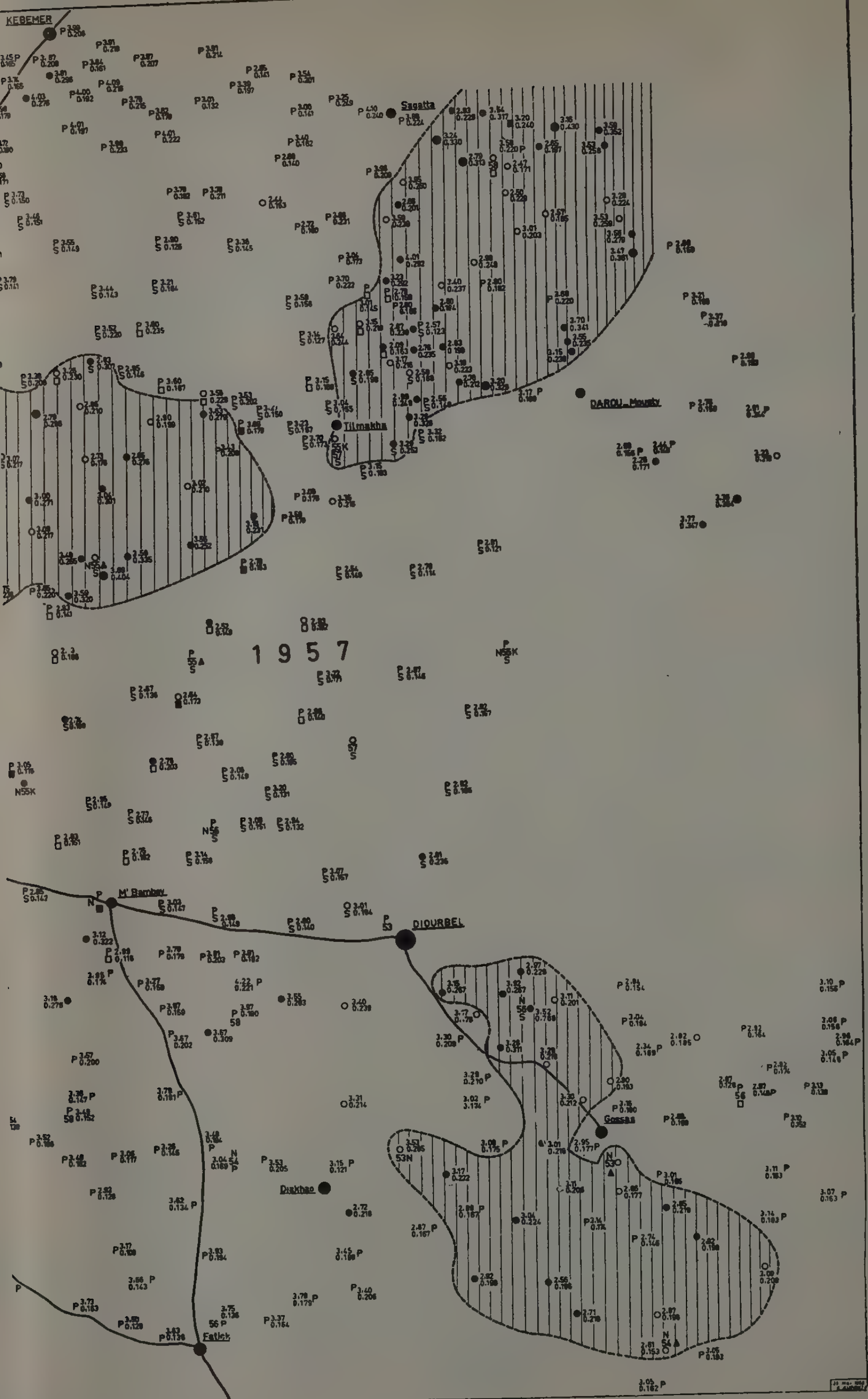


Figure (

Cartographie de la nutrition mi



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nérale par diagnostic foliaire.

a teneur en azote et sa teneur en phosphore)

TABLEAU III

Situation	Zone C
Détail	14 essais factoriels dans les régions de Thiès-Tilmakha et Gossas
Effet de N	+207 kg/ha
Effet de P	— 65 ”
Effet de K	+200 ”
Augmentation totale à l'hectare sans phosphore	+407 kg/ha

TABLEAU IV

Situation	Zone D
Détail	20 essais factoriels dans la région de Louga
Effet de N	+194 kg/ha
Effet de P	+124 ”
Effet de K	— 1 ”
Augmentation totale à l'hectare sans potasse	+318 kg/ha

PLACEMENT DE L'ENGRAIS

Les recherches sur les fumures au Sénégal ont montré l'efficacité de l'action de *faibles* doses de fumure minérale: 100 à 150 kilos d'engrais à l'hectare. Dans cette expérimentation, les engrais sont appliqués en side-dressing.

Des expériences de placement de l'engrais (Prevot et Ollagnier 1955) ont permis de conclure que l'application de faibles doses doit être localisée alors que, pour des doses plus élevées, l'application à la volée donne des résultats équivalents.

En voici un exemple où l'on voit que la supériorité de l'application en side-dressing par rapport à l'application à la volée s'atténue avec l'augmentation de la dose d'engrais: kilos de gousses supplémentaires à l'hectare pour application en side-dressing comparativement à l'application à la volée:

pour 50 kg d'engrais à l'hectare: +310 kg
pour 150 kg d'engrais à l'hectare: +156 kg
pour 220 kg d'engrais à l'hectare: + 0 kg.

Il faut remarquer que, dans les expériences, l'application à la volée de ces faibles doses est faite avec minutie pour obtenir une répartition homogène de l'engrais alors que, dans la pratique, le cultivateur ne prendra pas ces précautions. La localisation de l'engrais a donc l'avantage supplémentaire d'assurer une répartition plus uniforme de l'engrais. Les Services de Recherches de Nigeria sont d'ailleurs arrivés à la même conclusion après une expérimentation minutieuse (Greenwood 1950).

Mais l'application manuelle de l'engrais en side-dressing réclame beaucoup de temps, à une période où le cultivateur est déjà surchargé de travail. C'est pourquoi l'I.R.H.O. a mis au point un épandeur d'engrais qui peut être couplé au semoir (Figures 7 et 8). Après de nombreux essais et modifications réalisés depuis 1955, l'appareil est maintenant au point et a donné des résultats excellents tant au point de vue régularité de distribution que robustesse (Gillier 1959). Un calcul de rentabilité de l'appareil a montré que le prix de l'épandeur était remboursé dès la première campagne par le supplément de récolte dû au placement de l'engrais. Il est difficile de trouver des cas où la rentabilité d'un appareil est meilleure.

Figure 7

Epandeur d'engrais Fabre-Brevet I.R.H.O.

Figure 8

Semoir Fabre avec épandeur d'engrais permettant la localisation de part et d'autre de la ligne de semis.

FORMES D'ENGRAIS

La carence phosphorée étant très fréquente au Sénégal qui de plus possède d'importantes mines de phosphates, il était naturel d'axer l'effort principal sur la comparaison de diverses formes d'engrais phosphatés. Accessoirement, on a étudié l'action comparée du sulfate d'ammoniaque et de l'urée, ce qui a démontré l'existence de carences en soufre, et enfin l'action de diverses formes de soufre.

Phosphates

Nous avons vu qu'une zone du Sénégal ne présente pas de déficience en phosphore. Ceci n'a rien de surprenant, car cette zone est située au voisinage d'importants gisements de phosphate d'alumine et de phosphate tricalcique.

Le minerai est traité thermiquement dans un four rotatif puis broyé. Il donne un phosphate d'alumine calciné dosant 34 à 35% de P_2O_5 total dont environ 25% solubles au citrate d'ammoniaque. Il est commercialisé sous le nom de 'Phospal'.

Dans les comparaisons avec d'autres formes de phosphate, la teneur 34–35% de P_2O_5 total a été retenue pour le Phospal.

Le phosphate tricalcique, dont l'exploitation est actuellement arrêtée, était broyé finement (tamis 150) et commercialisé sous le nom de 'Baylifos'.

Enfin, dans la région de Taïba N'Diaye, un projet d'exploitation d'un gisement de phosphate naturel est en cours d'étude. Ces phosphates seront enrichis par un traitement par flottation, pour l'exportation. Le résidu, boues phosphatées à teneur en P_2O_5 assez faible, sera éventuellement disponible pour utilisation au Sénégal. Il est important pour l'économie de ce territoire de savoir dans quelle mesure est possible l'utilisation des ressources locales en phophates.

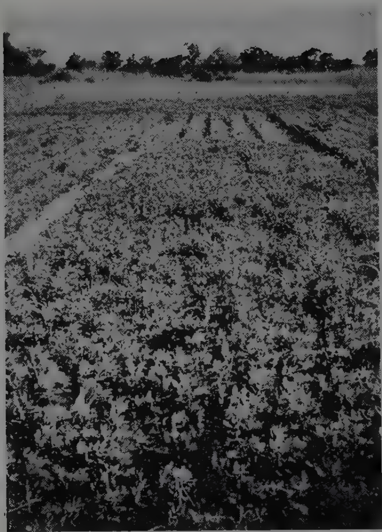
C'est la raison pour laquelle de nombreux essais ont comparé l'action du phosphate d'alumine calciné sur l'arachide.

Les nombreuses expériences réalisées par l'I.R.H.O. (Prevot et Ollagnier 1956) ont montré qu'à de faibles doses (15 à 60 kg de P_2O_5 /ha), le phosphate tricalcique n'a aucun effet notable ni sur la nutrition phosphorée, ni sur les rendements, en effet direct comme en effet résiduel. Le phosphate bicalcique a par contre un effet important et constant. Le Tableau V en donne un exemple.

TABLEAU V

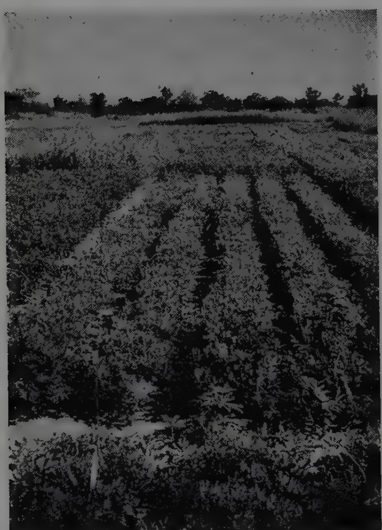
Effet comparatif des phosphates bicalciques et tricalciques

Stations		Effet du bicalcique	Effet du tricalcique	$\frac{\text{Bicalcique}}{\text{Tricalcique}}$
Tivaouane	1952	+210**	+10	+200**
Tivaouane	1953	+148*	+ 2	+146*
Bambey	1952	+370**	− 40	+410**
Bambey	1955	+400**	+ 40	+360**
Darou	1955	+440**	+160	+280**



(a) Sulfate d'ammoniaque = 3060 kg/ha

(b) Urée = 2130 kg/ha



(c) Témoin-rendement 2120 kg/ha

(d) Urée + sulfate de soude = 3030 kg/ha

Figure 9
Mise en évidence de l'effet du soufre.

L'étude a été faite sur l'ensemble d'un canton, mais n'a pas porté uniquement sur la vulgarisation des engrais. L'I.R.H.O. s'est efforcé de faire adopter par les cultivateurs africains un certain nombre de techniques très simples qui étaient susceptibles d'améliorer très fortement les rendements et dont la principale était l'utilisation des engrais. A cet effet, 28 blocs de 5 hectares ont été confiés à des cultivateurs avertis qui devaient exécuter les travaux selon les directives de l'I.R.H.O. Il leur était demandé:

(1) de respecter la rotation Arachide–Mil–Arachide–Jachère–Jachère (1 ha par phase).

(2) de séparer en 3 bandes les parcelles en arachide (1 bande sans engrais entourée de 2 bandes avec engrais).

(3) d'utiliser les semoirs à tractions animales munis de localisateurs d'engrais qui leur étaient distribués.

(4) d'employer les semences sélectionnées et désinfectées mises à leur disposition.

(5) enfin de semer à une densité voisine de 120.000 pieds/ha et d'entretenir normalement leur culture.

Alors que les rendements moyens de la région étaient de l'ordre de 800 à 1000 kg/ha, on a enregistré sur ces essais les résultats suivants:

Parcelle temoin	(bonne densité, graines desinfectées)	1250 kg/ha
Parcelle avec engrais	(bonne densité, graines desinfectées)	1780 "

Par ailleurs, dans une série d'essais de démonstration en culture traditionelle et sur 3 villages-témoins que l'I.R.H.O. suit de près depuis plusieurs années, l'effet moyen de l'engrais mesuré par échantillonnage avant la récolte a été:

Sur 39 hectares d'essais de demonstration	Sur 220 hectares fumés dans les villages-témoins
+ 840 kg/ha	+625 kg/ha

La validité des résultats de l'expérimentation est donc bien confirmée dans la pratique puisqu'on observe sur l'ensemble des cultures testées (soit sur 315 hectares) un effet moyen de l'engrais de 635 kg/ha alors que les essais scientifiques permettaient d'espérer un chiffre maximum de 880 kg/ha.

BIBLIOGRAPHIE

Gillier, P., 1959, Epandeurs d'engrais pour arachide, *Oléagineux*, **14**(5), 309–313.

Greenwood, M., 1950, The peasant use of fertilizers in Northern Nigeria, *E. Afr. Agr. J.*, **16**, 34–39.

Ollagnier, M. et Prevot, P., 1956, Comparaison du diagnostic foliaire et de l'analyse des sols pour la détermination des besoins en engrais de l'arachide, *Oléagineux*, **11**(6), 395–400.

PREVOT, P. ET OLLAGNIER, M., 1955, Localisation et granulation des engrais (Engrais organo-minéraux), *Oléagineux*, **10**(7), 489–493.

PREVOT, P. ET OLLAGNIER, M., 1956, Expérimentation sur les phosphates de la région de Thiès (Sénégal), *Oléagineux*, **11**(8/9), 545–549.

PREVOT, P., OLLAGNIER, M. ET FOURRIER, P., 1953, Carence azotée et phosphorée de l'arachide dans la region de Louga (Sénégal), Expérimentation 1952, *Oléagineux*, **8**(3), 135–138.

SAG, G., 1956, Influence de l'aluminium sur le développement de l'arachide, *Oléagineux*, **11**(5), 315–322.

THE EFFECT OF PURINE AND PYRIMIDINE BASES UPON THE SEASONAL CHANGES OF CHLOROPHYLL IN CITRUS TREES

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ABSTRACT

1. The effect of adenine, caffeine, uracil and xanthine upon the seasonal changes of chlorophyll were studied.

2. Adenine raised the chlorophyll level already a short time after its application. The level of chlorophyll in the adenine-treated trees remained significantly higher than that of untreated controls, throughout the growing season.

3. Caffeine, uracil and xanthine had no effect or caused an initial decrease in the chlorophyll level. Later in the growing season, these purines and pyrimidines started to stimulate the synthesis of chlorophyll which rised then significantly above the level of the control trees and reached the level of the adenine-treated trees.

4. There is no significant difference in the chlorophyll/DNA ratios obtained for treatments and control, at any of the sampling dates.

5. The results are discussed in the light of the hypothesis that there exist certain relationships between chlorophyll and DNA.

The reactions related to the aging of plants are as yet poorly understood. Many biochemical changes that are believed to occur in aging tissues, have been studied in excised tissues where the general trend has been found to be from anabolism to catabolism. One of the catabolized substances in detached leaves is chlorophyll. With regard to chlorophyll, it has been found that kinetin (Richmond and Lang 1957) and adenine (Kessler, in preparation) increased its stability in detached leaves, thus pointing to the possibility that aminopurines or their related nucleotids or nucleic acids might be related to the synthesis or to the stability of chlorophylls. The question now arises whether such relations between aminopurines and chlorophyll exist also in undetached leaves. This work deals with the effect of several purine and pyrimidine bases, which in previous work were found to affect nucleic acids in fruit trees and annual plants, upon the level of chlorophyll in citrus trees in the course of the growing season.

MATERIALS AND METHODS

For these studies 4-year-old Shamouti orange trees, budded on sour orange stock and growing on a medium textured, well aerated sandy-loam, were used.

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The trees were sprayed on March 31, 1958, with 150 ppm aqueous solutions of adenine (A) (Kessler 1956; Kessler and Lavee 1959; Kessler, Swirsky and Tahori 1959), caffeine (C) (Kessler and Lavee 1959; Kessler, Swirsky and Tahori, 1958, 1959), uracil (U) (Kessler 1955; Kessler, Bak and Cohen 1959; Kessler and Lavee 1959) and xanthine (X) (Kessler, Bak and Cohen 1959; Kessler and Lavee 1959; Kessler, Swirsky and Tahori 1958, 1959). Each treatment was replicated 4 times, in a randomized blocks outlay. Each replicate comprised 4 trees receiving about 2 litres each of the respective solutions. The solutions were prepared just before treatment, and a spreader (Triton) was added. Leaf samples for analyses were collected at 4 dates during the growing season. The first leaf samples were harvested on March 31 just before the application of the treatments. At this time the leaves reached about one third to half of their final size, and were smooth and light green. A second sample was taken on April 15, and a third on May 19. At the latter date the leaves had already reached their final size and their colour was deep green. A fourth sample of fully mature leaves was harvested on September 18.

ANALYTICAL METHODS

Chlorophyll: Chlorophyll was extracted with 96% ethanol from 5 g leaf samples. The colour intensity was then measured colorimetrically against a bichromate solution according to Guthrie (1928).

Total-N was determined by a micro-Kjeldahl method using a selenium-copper catalyst.

Alcohol-insoluble N: Dried material was extracted with 70% hot ethanol. The alcohol-insoluble fraction was then analysed by a micro-Kjeldahl method. This alcohol-insoluble nitrogen fraction is designated for convenience as "protein-N".

Nucleic acids: The plant material was first homogenized and soluble nucleotides were extracted 3 times in the cold with 0.2 N perchloric acid. The extraction was continued with a 1:3 mixture of ethanol–ether, ether–carbon tetrachloride and again with ethanol–ether. The nucleic acids were now extracted from the defatted tissue with 1 N perchloric acid for half an hour at 70°C (Kessler 1955, 1956). For further treatments the supernatant was used, and the insoluble fraction of this stage was discarded. The nucleic acid containing supernatant was now brought to *p*H 7.8 with KOH. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were fractionated for 20 hours at 37°C. This solution was now brought to *p*H 2 with perchloric acid, and under these conditions DNA precipitated. The RNA-mononucleotides containing solution and the DNA containing precipitate were separated by centrifugation.

The RNA in the supernatant was determined by the orcinol colour method (Markham 1955).

DNA was determined by the indole method as developed by Ceriotti (1952). For

this purpose the precipitated DNA was redissolved by bringing the solution up again to pH 6. After centrifugation the supernatant was used for the colour analysis.

The results were analysed by the Student-Newman-Keuls (Q) test (Federer 1955).

RESULTS

In Figure 1 the chlorophyll contents per fresh matter of the different treatments are plotted against time. It is seen from Figure 1 that within the first 6 weeks after the treatment there were no significant differences between the treatments and the untreated control, except for the adenine. Adenine had an immediate enhancing effect on the level of chlorophyll which is highly significantly different from the control trees as well as from the other treatments. The enhancing effect of adenine became apparent already a fortnight after its application. This difference between adenine and the other treatments became nonsignificant later in the season when all the tested purine and pyrimidine bases raised the chlorophyll level as compared to the control.

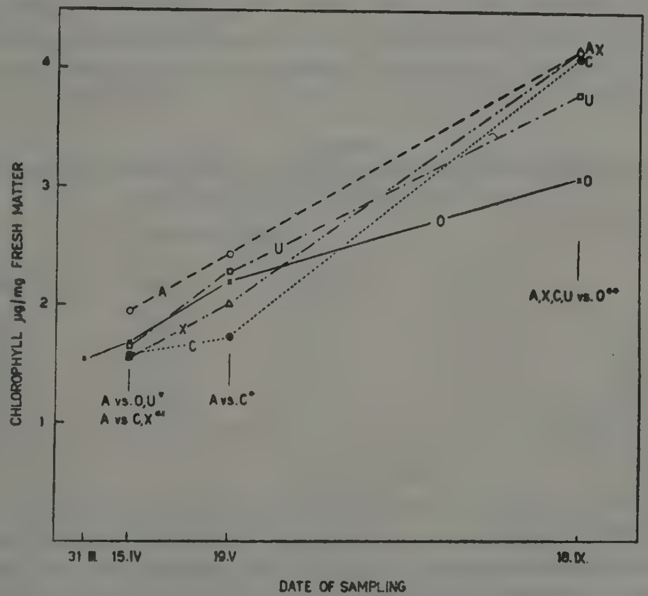

Figure 1

Chlorophyll content of Shamouti orange leaves on fresh weight basis, as affected by different treatments: 0 = control; A = adenine; C = caffeine; U = uracil; X = xanthine (Comparison significant at 0.05 level = *; comparison significant at 0.01 level = **; comparison not significant = N. S.).

The effect of adenine is even more pronounced if we base the chlorophyll level on the dry matter (Table I). It is seen that in this case the chlorophyll content of the control trees remained almost constant throughout the season while all the treat-

TABLE I

Chlorophyll content of Shamouti orange leaves, as affected by different treatments, on dry matter basis (µg/mg)

Treatment		Sampling dates			
		March 31	April 15	May 19	September 18
Control	(O)	7.50	7.27	7.27	7.35
Adenine	(A)	—	8.67	7.70	9.93
Caffeine	(C)	—	7.04	5.40	9.76
Uracil	(U)	—	7.33	6.69	8.97
Xanthine	(X)	—	7.18	6.24	10.27
Significance of comparisons [a]			A vs. O, C, U, X*	A, O vs. C*	A, C, X vs. O*

ments induced considerable changes in the chlorophyll level. Of particular interest is the fact that, apart from the immediate enhancing effect of adenine, the various purines and pyrimidines had either no effect, or they inhibited chlorophyll synthesis in the first 6 weeks after application of the treatments. This inhibiting effect is again shown, if we calculate the chlorophyll content per total, and especially per protein nitrogen (Table II and Figure 2). In all cases and at all sampling times, adenine

TABLE II

Chlorophyll content of Shamouti orange leaves, as affected by different treatments, on total nitrogen basis (µg/mg)

Treatment		Sampling dates			
		March 31	April 15	May 19	September 18
Control	(O)	242	285	320	356
Adenine	(A)	—	318	328	496
Caffeine	(C)	—	262	250	517
Uracil	(U)	—	288	300	457
Xanthine	(X)	—	260	291	543
Significance of comparisons [a]			N. S.	N. S.	X vs. O*

was promotive, although not always significantly so. But after 6 weeks there was a considerable increase in the level of chlorophyll in all treatments, and the autumn samples no longer differed significantly from each other, while they were all significantly higher than the control. The situation becomes somewhat different if chlorophyll is based on RNA (Figure 3). In the latter case the chlorophyll level of the adenine-treated trees remains significantly higher during the whole growing season, as compared with control and with other treatments. The differences between adenine and some of the other substances is apparent in this case also in the autumn, when

[a] See legend to Figure 1

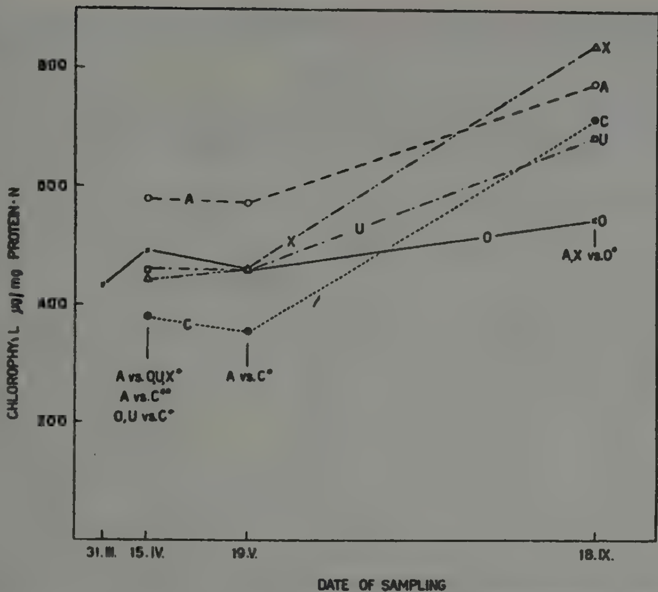

Figure 2

Chlorophyll content of Shamouti orange leaves on protein nitrogen basis, as affected by different treatments (as detailed in legend to Figure 1).

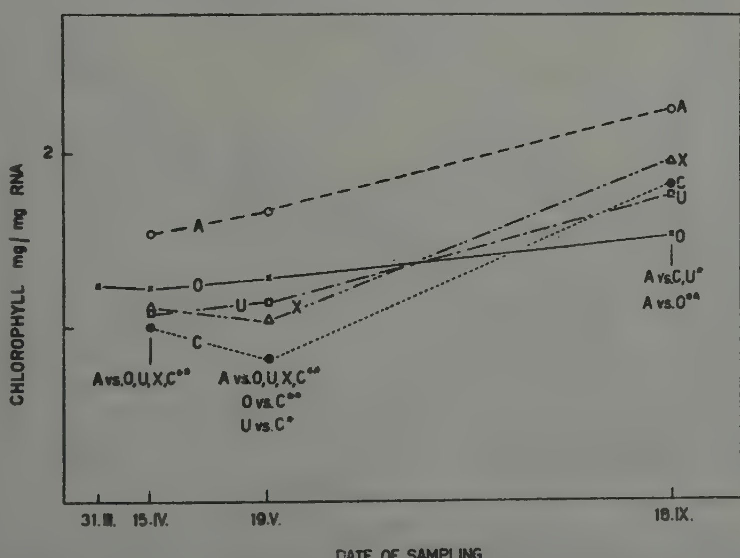

Figure 3

Chlorophyll content of Shamouti orange leaves on RNA basis, as affected by different treatments (as detailed in legend to Figure 1).

no differences were seen any more if chlorophyll was based on wet or dry weight, total or protein nitrogen. On the other hand, if we calculate the chlorophyll content per unit leaf area (Table III), no treatment, including adenine, was significantly different from the control, except in the autumn sample where all the treatments become significantly different from control, but not among each other.

TABLE III

Chlorophyll content of Shamouti orange leaves, as affected by different treatments, on leaf area basis ($\mu g/sq.\ cm$)

Treatment		Sampling dates			
		March 31	April 15	May 19	September 18
Control	(O)	44.5	49.0	69.3	102.7
Adenine	(A)	—	55.7	77.2	141.0
Caffeine	(C)	—	46.7	55.1	130.5
Uracil	(U)	—	49.8	71.3	125.0
Xanthine	(X)	—	49.8	63.1	146.7
Significance of comparisons [a]			N. S.	N. S.	A, X vs. 0** C, U vs. 0*

If we base the chlorophyll content on DNA (Figure 4) no significant differences were obtained between the treatments and the controls as well among each other,

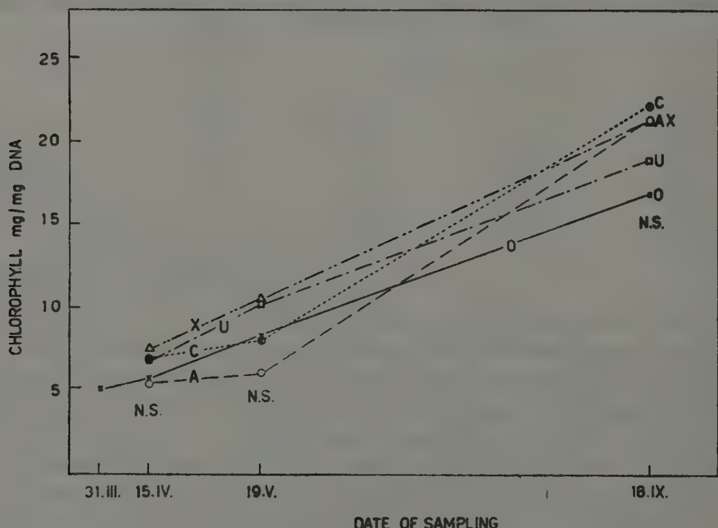

Figure 4

Chlorophyll content of Shamouti orange leaves on DNA basis, as affected by different treatments (as detailed in legend to Figure 1).

[a] See legend to Figure 1

and the chlorophyll/DNA ratio differences remained non-significant throughout the growing season.

DISCUSSION

In this work the chlorophyll content of Shamouti orange leaves has been calculated on various bases, and it appears that DNA is the most suitable one. If we assume the DNA content to be fixed per cell at time of our sample collections, it can be concluded that the chlorophyll level per cell increased almost linearly during the growing season, irrespective of treatment.

The results reported in this paper repeatedly demonstrate the considerable enhancing effect of adenine upon the synthesis of chlorophyll. Caffeine, uracil and xanthine, on the other hand, promoted the synthesis of chlorophyll only late in the growing season, whereas they had no effect or inhibited chlorophyll synthesis during the first weeks after the application of the treatments. An effect of aminopurines upon chlorophyll has already been shown for kinetin (Richmond and Lang 1957) and adenine (Kessler, in preparation) which were both found to increase the stability of chlorophyll and postpone its destruction in detached leaves. It was further found that, when applied in the early spring while the leaf meristems were still dividing, aminopurines actually stimulated chlorophyll synthesis in chlorotic fruit tree leaves (Kessler 1956). This stimulation was thought to be related to an increased DNA synthesis and its interaction with the so-called active iron fraction (Oserkowsky 1933) which was suggested to be involved in chlorophyll synthesis. The inhibition of DNA synthesis decreased the level of this active iron fraction (Kessler 1956) and lowered also the chlorophyll level, while the stimulation of DNA synthesis increased the active iron fraction as well as the chlorophyll level. In order to test these reported relations between chlorophyll and DNA, we plotted the chlorophyll and DNA levels against each other, irrespective of the treatment employed, and calculated the coefficient of correlation between both variables for each sampling date (April 15, $r = +0.881^{**}$; May 19, $r = +0.558^{*}$; September 18, $r = +0.542^{*}$). It follows that in Shamouti leaves, too, there exists a significant positive correlation between DNA and chlorophyll. Adenine, stimulating DNA synthesis (Kessler 1956), raised also the chlorophyll level (Figures 1, 2 and 3). Caffeine, on the other hand, inhibiting DNA synthesis (Kessler, Swirsky and Tahori 1958, 1959) in a way similar to mustard gas (Auerbach and Robson 1946, Herriott 1951, Kessler 1956), caused an initial decrease in the level of chlorophyll (Figures 1, 2 and 3). It thus seems that there exist, as already suggested in a previous report (Kessler 1956), certain relationships between chlorophyll and DNA, but these are of a rather complex nature. The coefficients of regression of chlorophyll on DNA are much lower in the April and May samplings than in the September one ($b_{\mathrm{April}} = +\ 3.00$; $b_{\mathrm{September}} = +\ 12.3$, the difference being significant at the 0.05 level). These phenomena are being studied more closely at present. The confirmation of a close relation between DNA and chlorophyll would be of particular interest, in view of the genetical implications of chlorophyll synthesis.

REFERENCES

AUERBACH, C. AND ROBSON, J. M., 1946, Chemical production of mutations, *Nature, Lond.*, **157,** 302.

CERIOTTI, C., 1952, A microchemical determination of deoxyribonucleic acid, *J. biol. Chem.*, **198,** 297.

FEDERER, W. T., 1955, *Experimental Design*, The Macmillan Co., New York, 544 pp.

GUTHRIE, J. D., 1928, A stable colorimetric standard for chlorophyll determination, *Amer. J. Bot.*, **15,** 86.

HERRIOTT, R. M., 1951, Nucleic acid synthesis in mustard gas-treated *E. coli* B., *J. gen. Physiol.*, **34,** 761.

KESSLER, B., 1955, Effect of methyltryptophan and thiouracil upon protein and ribonucleic acid synthesis in certain higher plants, *Nature, Lond.*, **178,** 1337.

KESSLER, B., 1956, Effect of certain nucleic acid components upon the status of iron, deoxyribonucleic acid and lime-induced chlorosis, *Nature Lond.*, **179,** 1015.

KESSLER, B., In preparation.

KESSLER, B., BAK, R. AND COHEN, A., 1959, Flowering in fruit trees and annual vegetables as affected by purines and triiodobenzoic acid, *Plant Physiol.* (in press).

KESSLER, B. AND LAVEE, S., 1959, The effect of purines, pyrimidines and metals upon the flowering of olive trees and grape vines, *Ktavim, Agric. Res. Sta. Rehovot*, **9** (in press).

KESSLER, B., SWIRSKY, E. AND TAHORI, A. S., 1958, The effect of caffeine and other purines upon the RNA/DNA ratio and nitrogen fractions of leaves and upon the suitability of these leaves to aphids, *Nature, Lond.*, **181,** 1595.

KESSLER, B., SWIRSKY, E. AND TAHORI, A. S., 1959, The effect of purines upon the nucleic acids and nitrogen metabolism of leaves and their sensitivity to aphids, *Ktavim, Agric. Res. Sta. Rehovot*, **9** (in press).

MARKHAM, R., 1955, Nucleic acids, their components and related compounds, In: *Modern Methods of Plant Analysis*, Paech, K. and Tracey, M. V. (Editors), IV, Springer-Verlag, Berlin, 246 pp.

OSERKOWSKY, J., 1933, Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves, *Plant Physiol.*, **8,** 449.

RICHMOND, A. E. AND LANG, A., 1957, Effect of kinetin on protein content and survival of detached *Xanthium* leaves, *Science*, **125,** 650.

ASPECTS OF THE TISSUE WATER RELATIONSHIPS OF AN IMPORTANT ARID ZONE SPECIES (*ACACIA ANEURA* F. MUELL.) IN COMPARISON WITH TWO MESOPHYTES

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ABSTRACT

Tissue water relationships of *Acacia aneura* F. Muell. in terms of relative turgidity, diffusion pressure deficit (DPD) and osmotic pressure were compared with those for two mesophytic species, tomato (*Lycopersicon esculentum* Mill.) and privet (*Ligustrum lucidum* Ait.).

A. aneura was shown to be much more resistant to desiccation than the other two species. In *A. aneura* a DPD of 15 atm reduced the relative turgidity to only 92 per cent whereas the same degree of water stress reduced the relative turgidity of tomato to 73 per cent and of privet to 87 per cent. In order to reduce the relative turgidity to 50 per cent a DPD of nearly 60 atm was required for *A. aneura*, 22 atm for tomato and 38 atm for privet. *A. aneura* tissue recovered from a DPD of approximately 130 atm without injury while DPD's of 45 and 90 atm proved lethal to tomato and privet respectively.

A feature of the DPD developed in *A. aneura* was that it could only partially be explained by increasing osmotic pressure whereas in the other species the DPD was satisfactorily explained on this basis.

INTRODUCTION

In a separate paper, Slatyer (1959) has described the methodology of a comprehensive water balance study conducted on an *Acacia aneura* F. Muell. plant community in central Australia. This contribution describes in more detail some of the internal water relationships of the species and compares them with data for two mesophytic species—privet (*Ligustrum lucidum* Ait.) and tomato (*Lycopersicon esculentum* Mill.)

The region in which the *Acacia aneura* community was located has an average annual rainfall of 10 in. (250 mm) of which three quarters is received in the summer six months, October-March and one quarter during the winter six months, April-September. In individual years however the rainfall is very sporadic and, on occasions, up to 90 per cent of the annual rainfall has been received in any two months of the year. Occurrences of three consecutive rainless months are common so that the plants frequently persist under conditions of extreme water stress for protracted periods.

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The *A. aneura* community is regarded as one of the most valuable of the arid zone plant communities in Australia because of its widespread distribution and the fact that it provides an extremely drought tolerant herbage of high feed value. The individual trees are low and shrubby reaching about 5-6 metres in height at maturity. As is the case with some other *Acacias* true leaves do not occur, except in the seedling stage, and the 'leaves' on the trees are actually phyllodes which are of the order of 1-3 mm wide, 1 mm thick and 3-10 cm long.

EXPERIMENTAL

For the purpose of the physiological measurements conducted in this experiment two groups of *A. aneura* individuals were examined in detail. These were arbitrarily characterised as mature and immature, the former being trees which had reached the average maximum height of the community and the latter those which were approximately half this height.

In addition, spot measurements were made on the complete size range of trees and on trees of similar size in different ecological situations. In order to provide further information, two treatments were imposed on the plants, one of which involved the provision of frequent (weekly) supplemental irrigation at a level which eliminated severe soil water stress, the other consisted simply of plants along the measurement transects which received natural rainfall alone. Thus the former set of plants were subjected only to atmospheric stress while the latter set were exposed to both atmospheric and soil water stress. Because the supplementary irrigated plots were small and the soil surface was dry before measurements were made (irrigation took place two days prior to sampling) the effect of the irrigation on the micro-climate around the artificially watered plants was negligible.

Three main water relations measurements were made—of phyllode water content (as relative turgidity), diffusion pressure deficit (DPD) and osmotic pressure. In addition, transpiration estimates were available from soil water balance data and growth was measured as shoot elongation.

Relative turgidity measurements were made in a manner similar to that described by Weatherley (1950). Phyllodes were plucked from the top branches of the sample trees and immediately separated into duplicate samples of 10 phyllode segments each 1.5 cm long. The samples were then weighed (fresh wt.), floated on distilled water for 24 hours, surface dried with filter paper using a standard drying procedure, reweighed (turgid wt.), dried to constant weight at 80°C and reweighed (dry wt.). Relative turgidity was obtained by:

$$\text{Fresh Wt. - Dry Wt. / Turgid Wt. - Dry Wt.}$$

and expressed as a percentage.

The floating stage took place in a refrigerator at 5-7°C. This procedure was adopted to minimise metabolism and cell growth so that the water uptake observed would reflect only the passive uptake needed to satisfy the water deficit in the tissue. Also the amount of uptake was directly influenced by fluctuations in water temperature (Werner 1954) and a refrigerator provided the only constant temperature facility available.

Diffusion pressure deficit (DPD) was measured using the vapour equilibration technique of Slatyer (1958). Sampling procedure and sample size were identical with those used for relative turgidity determinations.

The osmotic pressure of the tissue fluids was determined cryoscopically on the expressed sap. Samples of tissue identical with those used for DPD and relative turgidity determinations were wrapped in aluminium foil and snap frozen to -20°C. They were then thawed and the sap expressed at 6000 lb/in^2.

Dawn was found to be the most significant time for plant sampling in this area since, at this time, the internal water balance was virtually static and day to day comparisons could be drawn. At other times of the day the flux in the water relations quantities prevented direct comparative data from being obtained. Moreover, at dawn tissue hydration was at a maximum for the whole day; this was of direct significance to growth and general metabolism. All data presented were obtained from samples taken at dawn.

Data for privet and tomato were obtained from a laboratory experiment conducted at Duke University, U.S.A. The general details of this experiment are reported elsewhere (Slatyer 1957), the same techniques being employed for the various measurements as were used for *A. aneura*.

RESULTS

In Figure 1 data for DPD of *A. aneura* are plotted against relative turgidity (Curve 1) and for osmotic pressure against relative turgidity (Curve 2). Curve 1 represents the accumulated relative turgidity and DPD data from six months of continuous weekly sampling, plotted for mature and immature groups of trees and for naturally and artificially watered treatments.

It can be seen that all of the points fall close to the fitted curve and that there is no evidence of the different treatments exhibiting a different relative turgidity/DPD relationship. This is of interest because the naturally watered and artificially watered treatments represented two extreme aspects of pre-conditioning and it was anticipated that changes in the physiological character of the tissue may have occurred. Any such changes could readily have been reflected in different relative turgidity/DPD relationships; particularly if the osmotic pressure of the cell sap was affected (excluding the direct influence of decreasing hydration of osmotic pressure) since this value is of primary importance in determining the form of the relative turgidity/DPD relationship. However almost all of the tissue sampled from the artificially watered

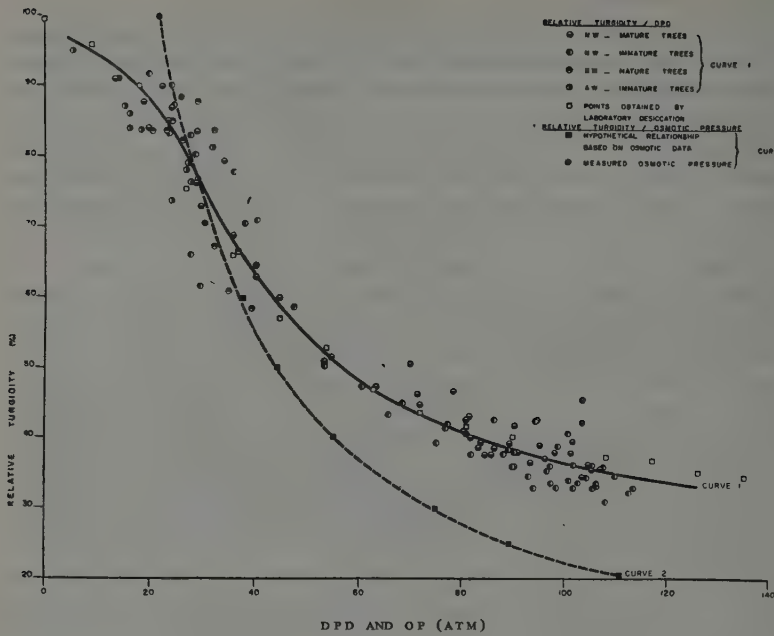

Figure 1

Relationship between relative turgidity, DPD and osmotic pressure in phyllode tissue of *A. aneura*. *Curve* 1 (Solid line) - Relative turgidity/DPD relationship for tissue dehydrated under natural conditions (circles) and in the labotarory (hollow squares).

Curve 2 (Broken line) - Relative turgidity/osmotic pressure relationship derived from actual measurements (solid circles) and estimation (solid squares).

trees had already formed when the treatment was first applied and this may have limited the extent of alteration in physiological constitution. Certainly anatomical changes were of a minor nature.

A number of points have been added to Curve 1 to provide further evidence as to the osmotic relationships of the tissue during natural dehyration. These points were obtained by floating tissue (originally at about 80 per cent relative turgidity) to full turgor and then dehydrating it in a range of micro-desiccators of different vapour pressures. Thus the values shown were obtained by rapid (48 hours) dehydration of tissue in micro–desiccators rather than by the slow dehydration which took place under natural conditions. From the diagram it is apparent that the points

fit satisfactorily on the original curve. Since the rapid dehyration would have minimised any physiological changes in the composition of the cell sap the results support the contention that such changes during natural dehydration were of a minor nature.

The shape of Curve 1 is also of special interest as it provides an opportunity to determine how closely the behaviour of *A. aneura* tissue approximated that expected from normal vacuolated tissue characteristic of mesophytes. Curve 2 has been drawn to describe the behaviour expected from the tissue if the DPD was determined solely by changes in the osmotic pressure of the tissue fluids induced by the direct effect on concentration of decreasing water content.

This curve was derived from osmotic pressure determinations made on the turgid material used for the laboratory desiccation just decribed and from separate osmotic pressure determinations. The osmotic pressure of the expressed sap from the tissue

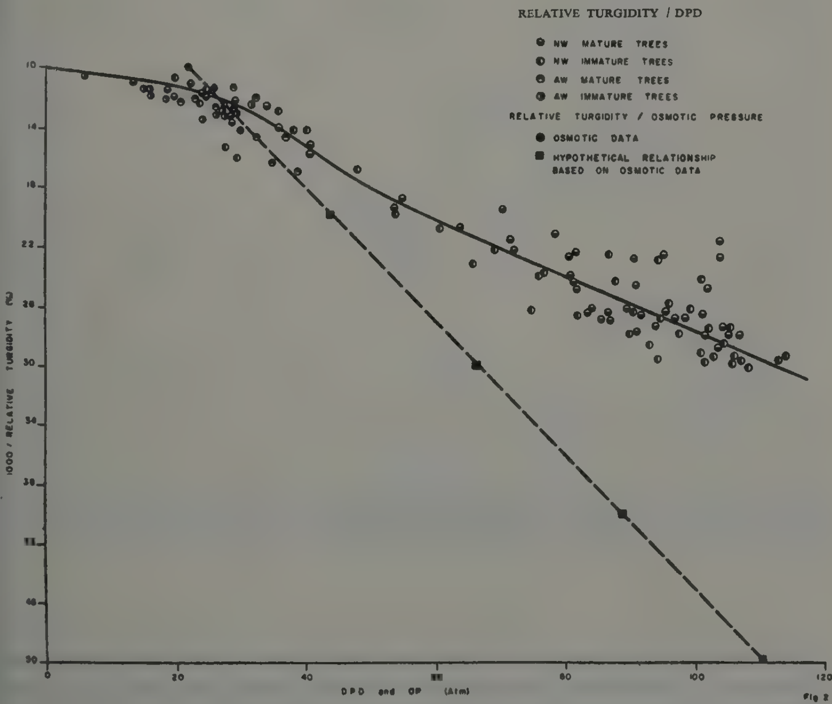

Figure 2

DPD and osmotic pressure data from Figure 1 plotted against reciprocals of relative turgidity determinations.

at full turgor was found to be 22.3 atm. If the tissue was composed of thin flexible walled cells which were fully vacuolated it would be anticipated that at approximately 28 atm DPD there would be zero turgor pressure in the tissue and as further dehydration occurred the DPD would develop solely as a result of changes in osmotic pressure.

The curve reveals the extremely interesting result that this hypothetical relationship diverged gradually from the measured relationship of Curve 1 until, at 120 atm DPD,

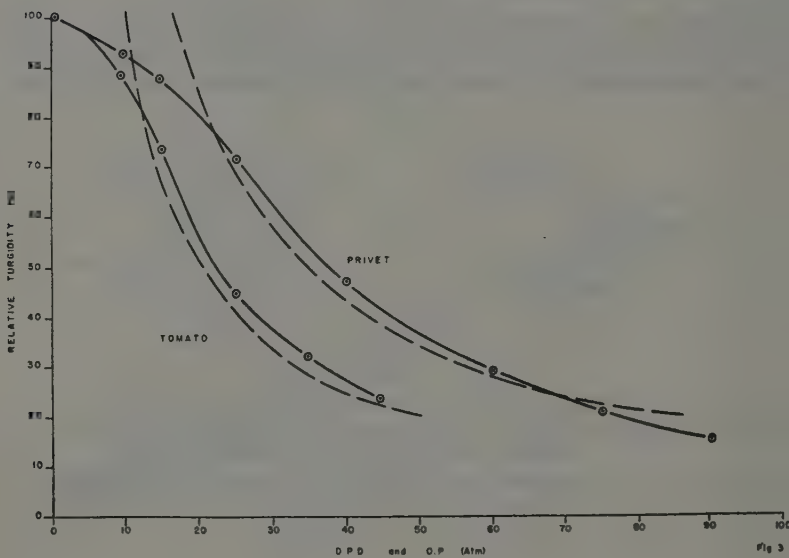

Figure 3

Relationship between relative turgidity, DPD and osmotic pressure in leaf tissue of privet and tomato. Solid lines refer to relative turgidity/DPD relationship; broken lines to relative turgidity/osmotic pressure relationship.

it represented a relative turgidity value 15 per cent lower. In order to examine this feature in more detail, Figure 2 has been constructed by plotting the DPD and osmotic pressure data of Figure 1 against a reciprocal of relative turgidity. In this way the exponential relationship of relative turgidity and osmotic pressure appears as a straight line.

From the figure the divergence of the two curves is seen to be even more pronounced. Furthermore, the portion of the relative turgidity/DPD curve at DPD's in excess of about 40 atm is also fitted by a straight line. At lower DPD's when positive turgor pressure was operative, the curve deviates from a straight line. On the one hand this suggests that a measurement error in determination of osmotic pressure was possibly responsible for the divergences of the two curves, but the data themselves eliminate this possibility since a measurement error of about 100 per cent

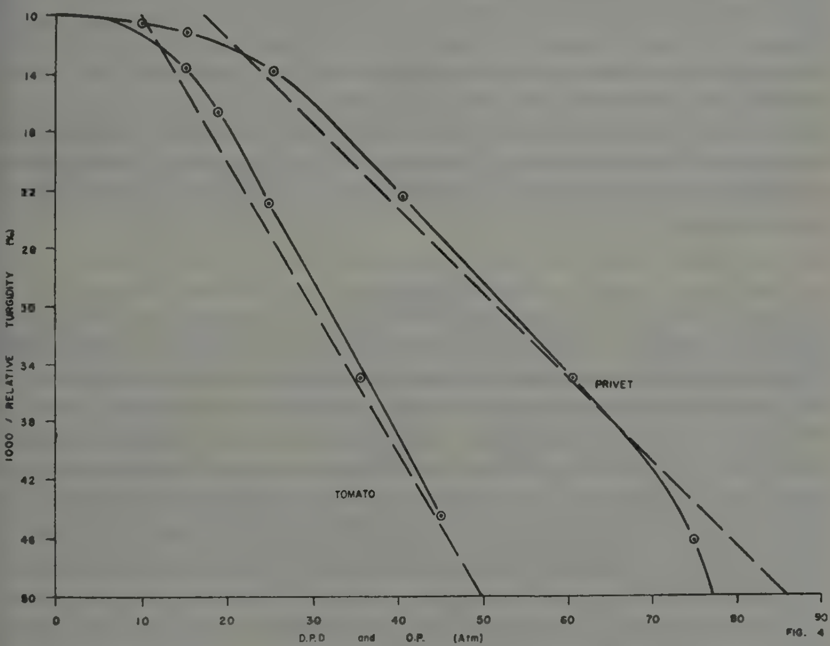

Figure 4

DPD and osmotic pressure data from Figure 3 plotted against reciprocals of relative turgidity determinations.

would be involved and the replicated determinations were considered accurate to better than $\pm$ 10 per cent.

Assuming then that this difference was real, it appears that additional factors were operative in the development of DPD under conditions of extreme desiccation. This possibility will be considered in the subsequent discussion.

In order to compare these features of the water relationships of *Acacia aneur* with other species, data for two mesophytes are presented in Figures 3 and 4 below The two species selected for study were tomato and privet. While tomato is readil classified as a mesophyte, the privet used, a frost hardy shrub from the America piedmont, has some xeromorphic characteristics such as thickened cuticles, an was shown by Weatherley and Slatyer (1957) to be more tolerant of desiccation tha tomato.

However for both species the relative turgidity/DPD and relative turgidity/osmo tic pressure curves (Figure 3) showed little divergence except when positive turgo pressure was of influence in determining the DPD. When the data are plotted agains reciprocals of relative turgidity (Figure 4) the same pattern was repeated, littl divergence being evident and the closely parallel lines suggesting that in these specie the DPD at higher stress levels was adequately explained by increase in osmoti pressure. In each case however, the paired lines for each species did intersect, presu mably at the point of zero turgor pressure. This may indicate the development o a slight negative wall pressure at higher DPD's in addition to the effect caused b osmotic pressure.

DISCUSSION

One of the most interesting features of the comparative relative turgidity/DPI data presented was the marked differences which existed between the species as t the degree of water stress which was required to reduce the tissue water content t certain levels. In *A. aneura* for instance a DPD of 15 atm only reduced the relativ turgidity to 92 per cent and a DPD of nearly 60 atm was required before the relativ turgidity fell to 50 per cent. Comparative data from tomato and privet showed tha a DPD of 15 atm reduced the relative turgidity to 73 and 87 per cent respectively; relative turgidity of 50 per cent in these species was induced by a DPD of 22 an 38 atm respectively.

It is apparent that the tolerance to desiccation exhibited by *A. aneura* is directl related to its extreme drought resistance. In addition, it can be mentioned that th end points of each of the relative turgidity/DPD curves represented the mos extreme desiccation to which the species were subjected during the course of th investigations. In the case of tomato and privet this was the most extreme DPI which the tissue could withstand and still recover when the stress was relieve The fact that in *A. aneura* DPD values of 130 atm could be tolerated, whereas i tomato and privet DPD's of 45 and 90 atm respectively caused the death of th tissue, is further evidence of the drought tolerance of the former species.

Some investigators have observed an increase in osmotic pressure during dehydra tion which is greater than can be directly explained by change in concentratio following dehydration. Ahrns (1924) and Spoehr and Milner (1939) have attribute

such phenomena to the breakdown of organic complexes to osmotically active substances during onset of drought. In the present study however the relative turgidity/DPD relationships of artificially dehydrated *A. aneura* tissue appeared to be almost identical with those of naturally dehydrated tissue, even though the period of natural dehydration frequently exceeded two months. This result depended for its validity on the absence of significant changes in the osmotic characteristics of the artificially dehydrated tissue during the 48 hour period required for equilibration. In general it appears that if such carbohydrate breakdown is to occur a considerably longer period is required; for instance Slatyer (1958) did not observe any significant changes in a series of laboratory desiccations on several species even though dehydration periods of up to 72 hours were used.

The development during dehydration of a DPD in *A. aneura* greater than that which could be expected on the basis of increased osmotic pressure is of particular interest. In both privet and tomato the increase in DPD appeared to be determined almost entirely by changes in osmotic pressure; at no time did the DPD exceed the osmotic pressure by more than 5 atm. This was also noted by Slatyer (1957) in a separate study of the same species.

In *A. aneura* however the differences became progressively more marked and appeared to be well in excess of any reasonable measurement error. This feature can possibly be partly explained on the basis of development of negative wall pressure, but it is hard to visualise this factor contributing more than 5-10 atm to the DPD. It would seem that the magnitude of the negative pressure which can develop in this way must be limited by the degree of adhesion between the protoplast and the cell walls. The highest measured values known to the author (10 atm) are those of Buhmann (1935).

Since the relative turgidity/DPD curve of *A. aneura* is also exponential in form, it seems more probable that the increase in DPD may be due to a factor similar in action to osmotic pressure. In this context an imbibitional factor could possibly be of significance, since the cell walls and vacuoles of arid zone plants contain hydrophilic colloids with pronounced imbibitional properties (Roberts and Styles 1939, Hofmeister 1940, Gortner, Gortner and Gortner 1949). At the present time the evidence is inconclusive but the phenomenon will be the subject of further study.

REFERENCES

Ahrns, W., 1924, *Bot. Arch.*, **5**, 234.

Buhmann, A., 1935, *Protoplasma*, **23**, 579.

Gortner, R.A., Gortner, R.A., Jr. and Gortner, W. A., 1949, *Outlines of Biochemistry*, Wiley, New York.

Hofmeister, L., 1940, *Protoplasma*, **35**, 65.

Roberts, O. and Styles, S.A., 1939, *Sci. Proc. roy. Dublin Soc.*, **22**, 119.

Slatyer, R.O., 1957, *Austral. J. biol. Sci.*, **10**, 320.

Slatyer, R.O., 1958, *Austral. J. biol. Sci.*, **11**, 349.
Slatyer, R.O., 1959, *Proc. UNESCO Symp. Arid Zone, Plant Water Relations* (in press).
Spoehr, H.A. and Milner, H.W., 1939, *Proc. Amer. phil. Soc.*, **81**, 37.
Weatherley, P.E., 1950, *New Phytol.*, **49**, 81.
Weatherley, P.E. and Slatyer, R.O., 1957, *Nature*, **179**, 1085.
Werner, H.O., 1954, *Nebraska agr. Exp. Sta., Res. Bull.*, **176**.

MATERIALS FOR A REVISED FLORA OF PALESTINE. II *

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ABSTRACT

This paper reports on some results of the author's studies in the Kew Herbarium. It concerns several dubious species of the Flora of Palestine, as well as some rare or adventitious species not previously recorded from this country.

Cuscuta cesatiana Bertol. Fl. Ital. VII: 623. 1847.

Huleh Plain near Lehavoth Habashan, alluvial soil, paras. on *Epilobium* 6.7.1954 . Nahmoni; Esdraelon Plain, Afula, paras. on *Amaranthus lividus* 31.5.1955 . Frank. New for Palestine and possibly adventitious.

Rubia

In the second edition of Post's Flora (1932) six species of *Rubia* are recorded from alestine: *R. aucheri* Boiss., *R. tinctorum* L., *R. peregrina* L., *R. tenuifolia* D'Urv. omprising var. *stenophylla* Boiss. and var. *eburnea* Nab., and *R. velutina* Nab. his list should be restricted to two species only: *R. tinctorum* L. and *R. olivieri* . Rich. (= *R. tenuifolia* D'Urv.).

R. tinctorum L.

This species has been collected in Petra by Aaronsohn and in Alji near Petra y Nabelek. *R. peregrina* mentioned by Hart (1891) is apparently also *R. tinctorum* L.

R. olivieri A. Rich.

This is one of the commonest climbers of our flora. It displays considerable ariability in size and shape of leaves, in pubescence and scabrosity of branches nd in general habit. The number of leaves in a whorl varies from 4 to 6, often n the same specimen. The variability of the species is, however, not correlated ith ecological or geographical diversity.

Boissier (1875) subdivided *R. olivieri* into var. *stenophylla* Boiss. and var. *elliptica* oiss. The latter had been previously (1849) described by Boissier as *R. brachypoda*, ut was later reduced to a variety of *R. olivieri*. There is no doubt that *R. brachypoda* annot be upheld as a separate species. Moreover, even var. *elliptica* and var. *stenohylla* can hardly be accorded varietal rank. Specimens of *R. olivieri* display every

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* Materials for a revised flora of Palestine I. *Proc. Linn. Soc. London*, Sess. 157, 1944–45, Pt. 1, Dec. 1945.

eceived January 4, 1960.

transition in leaf shape, from elliptical to linear, making classification of specimen into varieties most difficult. *R. olivieri* also shows variability in the degree of scabro sity and pubescence of its branches.

Nabelek (1923) described *R. olivieri* var. *eburnea* Nab. from a specimen collecte by him at Alji near Wadi Musa and *R. velutina* Nab. collected among rocks at Wad Bet Hanina near Jerusalem. The distinctive diagnostics of var. *eburnea* are white striate shining branches and linear, 2 mm wide leaves. *R. velutina* he distinguishe from *R. olivieri* "caulibus velutinis, foliis quaternis non senis, corollis maioribu antheris subglobosis nec ovatis". Specimens of *R. olivieri* which correspond to th descriptions of var. *eburnea* and *R. velutina* can be found in the Judean Mountain especially in the sunny Batha and among rocks where the plants are exposed t intense light and often subjected to damage by browsing animals. In such habitat *R. olivieri* presents much wider variability in general appearance than in the shad of Maquis. In the Batha or among rocks it usually develops a more shrubby habi and the leaves are mostly borne on short shoots (brachyblasts), springing fror hardened, oft white, branches.

Observation of *Rubia* in all Mediterranean parts of the country leaves no doub that only one species of *Rubia*, *R. olivieri*, is represented here. *R. velutina* is therefor to be regarded as synonymous with *R. olivieri*, and *R. olivieri* var. *eburnea* scarcel as a distinct form.

Scilla hanburii Bak.

This species does not occur in Palestine. It has been described from Antilebanor and is apparently a species of subalpine altitudes. Dinsmore in the second editio of Post's Flora recorded it from Wadi Kilt in the Judean Desert.

In the Kew Herbarium the type specimen of Baker collected by Hooker an Hanbury has been compared by me with Dinsmore's specimen from Wadi Kil The sheet of the type specimen of *S. hanburii* comprises two conical fruiting inflores cences. One inflorescence is 7 cm long to 8 cm broad, the other 4 cm long to 5 cr broad; the pedicels are horizontally spread and 2–4 cm long in both. The fruitin inflorescence of Dinsmore's specimen from Wadi Kilt is cylindrical, 9–13 cm lon to $3\frac{1}{2}$–$4\frac{1}{2}$ cm broad; its pedicels are obliquely divaricate and arcuate. Dinsmore' specimen apparently belongs to *S. autumnalis* or to a desert variety of this specie *S. hanburii* has been collected in fruit on 9th October, Dinsmore's specimen on 28t November.

Since Dinsmore's is the only record of *S. hanburii* from Palestine, this species ha to be cancelled from the list of Palestinian species.

Muscari pulchellum Heldr. et Sart. in Boiss. Diagn. II.4.109. 1859.
Syn. *M. racemosum* auct. fl. pal.

M. racemosum appears in all sources dealing with the Flora of Palestine. Post (1896) records it as *M. racemosum* L. as "common from lower mountain zones to alpine regions and interior plains; Palmyrene Desert". Var. *stenophyllum* Post with leaves 1 mm broad is described from Amanus Mts. In the second edition of Post's Flora (1933) it is given as *M. racemosum* (L.) Mill. and is recorded from various localities in Palestine, Lebanon, Syria and Sinai. Eig (1933) records it as *M. racemosum* (L.) Lam. et DC.

Turrill (Curtis's Bot. Mag. 167. 124. 1950) showed that *M. racemosum* Mill. is synonymous with *Muscari muscarimi* Medic., and is quite different from *M. racemosum* (L.) Lam. et DC. *M. racemosum* should best be declared a *nomen confusum*. According to W. T. Stearn (personal communication) the correct name for the European plant usually known as *M. racemosum* is *M. atlanticum*.

Our plant differs from the European *M. racemosum* mainly by its colour and the density of its raceme, both characteristics obliterated in dried specimens.

M. pulchellum Heldr. et Sart. has been described from Greece but its area extends eastward, to Asia Minor and Transcaucasia, and southward to Palestine. The flowers of *M. pulchellum* are described by Boissier (1884) as "caeruleo-violacei". The colour is actually dusky-violet in the fertile flowers and blue in the sterile upper ones. This difference in colour of the fertile flowers distinguishes *M. pulchellum* from the European *M. racemosum* which has sky-blue flowers of a pure tinge and belongs to the group of light coloured *Muscari* species, like *M. armeniacum*. *M. pulchellum* like *M. racemosum* possesses white teeth of perigonium. The raceme of *M. pulchellum* is not as dense as that of *M. racemosum*, in which flowers appear imbricated.

The following specimens of *M. pulchellum* were examined. Asia Minor: Rankoei, ad radic. mont. Kara-Jun, P. Sintenis, Iter troj. 1883 N 1229 (det. Ascherson); Amasia, in pasc. montanis (Mt. Keklar), 6-800 m. 5.3. 1889 Bornmüller. Syria: Dera', fields, 550 m, 26.2.1912 Meyers et Dinsmore G 4371; Baalbek — Zebdani 22.4.1878 Post. Lebanon: Mt. Lebanon, Mitchell; heights of Bludan, 23.4.1878, Post. Palestine: Jerusalem Col. White 1917 (sub *M. neglectum* Guss.); Tel-Arad (Judean Des.), 24.3.1929 Gabrielith; Jerusalem 21.2.1931 Pl. Pal. Exs. 117 sub *M. racemosum*.

In Palestine *M. pulchellum* grows in the dwarf-shrub associations of the Mediterranean territories and in the northern steppe parts of the Negev. It is mainly an East-Mediterranean species, whereas *M. racemosum* is an Euro-Siberian one.

Asparagus lownei Bak.

Baker (1875) described this species from a specimen collected by Lowne near Jericho. In the Kew Herbarium I compared the type specimen of Baker's plant with his type specimen of *A. palaestinus* Baker from Huleh collected by Hayne. The cladodia of *A. lownei* are somewhat longer than those of the specimen of *A. palaestinus* from Huleh. However, observation of numerous specimens (in herbarium and

in vivo) of *A. palaestinus* from various parts of the country, comprising the banks of Jordan near Jericho, shows that the length of cladodia varies considerably in *A. palaestinus*. Since *A. lownei* does not differ from *A. palaestinus* in any significant diagnostic character, nor in its habitat, it seems justified to regard it as synonymous with *A. palaestinus* Baker. *A. palaestinus* is endemic to Palestine.

Platanthera holmboei Lindl. fil. Soc. Sc. Fenn. Arsbok 20 B No. 7 (1942) Figure 7 in Iter Cyprium Acta Soc. Sc. Fenn. N. Ser. B 2 No. 7. 1946.

Upper Galilee, Mt. Meron near Beit-Jann, maquis 27.4.1956 leg. T. Doobinsky.

A new genus for the flora of Palestine. This plant known as endemic to Cyprus has been found here for the first time.

Orchis punctulata Stev. ssp. *sepulchralis* (Boiss. et Heldr.) Soo

Mt. Carmel, Khreibe, chalky slope, Batha, 28.3.1956 leg. A. Shifman.

This plant has never been recorded from Cisjordania. Post (1933) records both the type and ssp. *sepulchralis* from Transjordan. However, the type form of *O. punctulata* seems to be confined to more northern latitudes (Crimea and W. Transcaucasia), whereas ssp. *sepulchralis* is an East-Mediterranean plant recorded from Thracia, the Aegaeis and Asia Minor; it apparently is the form which Bouloumoy (1930) recorded under *O. punctulata* from near Beirut (Lebanon).

SOME PROBLEMS IN DROUGHT RESISTANCE

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ABSTRACT

1. A terminology is suggested for eliminating some misunderstandings.
2. In the several plants tested, avoidance was always found to be the cause of drought resistance, except when frost hardening resulted in drought resistance, which proved to be tolerance.
3. A scheme is proposed for testing plants in order to determine whether their drought resistance is due to avoidance or tolerance and to what component of each.
4. A simple chamber for measuring drought resistance is described.

Though the adaptation of plants to drought has been studied for over a century (Oppenheimer 1959), many problems await further investigation before they can be solved. Some of the problems, on the other hand, are more apparent than real. Thus the great variety of adaptations associated with the growth of plants under conditions of drought (thoroughly and clearly described by Oppenheimer), is matched by the variety of terminology used by different investigators. This confusion in terminology leads to many misunderstandings that can be eliminated only by the adoption of a uniform and highly specific usage of terms. Table I attempts to solve this problem.

What, for instance, is the relation between xerophytism and drought resistance? According to Table I, every xerophyte must be drought-resistant, for unless it can survive a dry climate, it certainly cannot grow and develop in it. Yet drought resistance is not in itself able to endow a plant with xerophytism. The plant must also possess those properties that enable it to grow and complete its life cycle in a dry climate. Conversely, non-xerophytes may conceivably possess drought resistance, provided it is not accompanied by the ability to grow and complete their life cycle in a dry climate. This is, indeed, found to be the case since frost resistant plants are

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TABLE I

Classification of plants adapted to dry climates

Adaptability to dry climates

Xerophytism

Ability to stay alive

Drought resistance

Ability to prevent reduction in water content

Drought avoidance

Ability to complete life cycle before extreme drought

Ephemerals

(Drought escaping)

Ability to obtain large amount of water during drought

Water spenders

(Drought evading)

Ability to reduce water loss to a minimum

Water savers

(Drought enduring)

Ability to survive reduction in water content

Drought tolerance

(Drought hardiness, dehydration or desiccation resistance)

Ability to grow and develop (no name):

1. Ability to germinate
2. Suitable optimum temperature for growth
3. Suitable photoperiodic response
4. Suitable thermoperiodic response, etc.

also drought tolerant, yet many, at least, are not considered to be xerophytes. But it also follows from this classification that drought resistant plants may not be frost resistant provided that they owe their resistance to avoidance.

Another problem that is automatically solved by this classification is the relative importance of drought avoidance and drought tolerance in xerophytism. By definition, the factors responsible for drought avoidance help to maintain the optimum water content for growth. On the other hand, factors favouring drought tolerance permit survival at a cell water content too low to permit growth. In fact, frost tolerance (and therefore the correlated drought tolerance) is at its maximum when the plant is in the rest period and therefore unable to grow. Since, by definition, the ability to grow in a dry climate is one characteristic of xerophytism, it is obvious that drought avoidance should be a much more common property of xerophytes than drought tolerance. This is actually the case (Oppenheimer 1959).

There are, of course, all degrees of xerophytism, and a moderate degree of this property is essential for the survival of many economically important plants even in climates that cannot be considered dry in comparison with the deserts inhabited by the extreme xerophytes. It might, therefore, be suspected that drought tolerance may play a much more important role under these conditions. Thus soybean plants become resistant to drought as a result of temporary wilting. This is obvious from the fact that after a few days of this treatment they recover from the wilting without the addition of water. But this is due to a reduced transpiration rate and they show no increase in drought tolerance (Clark and Levitt 1956). Similarly, two oak species adapted to different water regimes fail to show any difference in drought tolerance (Sullivan and Levitt 1959). These two sets of results seem to indicate that even in the case of moderate drought resistance, avoidance is more important than tolerance. In an attempt to find out how widespread this phenomen is, the drought tolerance of a number of plants in the unhardened and hardened states was determined.

Drought tolerance was measured by allowing sections to come to equilibrium with a series of relative humidities, in order to determine the relative humidity that caused approximately 50% killing of the cells. The method is described in detail in the above references. The plants were drought hardened by watering only when the plants were severely wilted, and continuing this treatment for 2–6 weeks. As in the case of soybeans (Clark and Levitt 1956), many of the plants treated in this way showed their adaptation to drought by recovering from wilting after a few days' treatment without the addition of water. The control plants were watered often enough to prevent any wilting.

The results (Table II) indicate that plants known to be adapted to drought (millet and *Sempervivum glaucum*) fail to undergo any detectable increase in drought tolerance. On the other hand, plants that are not usually considered to be as xerophytic as these (cabbage, wheat, barley) showed a significant increase in tolerance. These latter are capable of becoming frost tolerant, and Table III shows that the frost hardening process is just as effective in inducing drought tolerance as frost tolerance.

TABLE II

Drought tolerance of unhardened plants and of plants hardened by droughting for 2—6 weeks

Species	Relative humidity causing 50% killing	
	Unhardened	Hardened
Millet	92	92
Cabbage (*Brassica oleracea*)	96	92
Sempervivum glaucum	94	94
Wheat (Thorne)	98	98
Wheat (Kharkov)	98—93	94—93
Wheat (Seneca)	97	94
Barley (B475) long-day	96	90
Barley (B475) short-day	96	94

TABLE III

Drought hardiness of unhardened barley plants and of plants hardened by exposure to low temperatur

Variety	Relative humidity causing 50% killing	
	Unhardened	Hardened
B475	98	90 (Grown outdoors)
	94	90 (Greenhouse-grown
Tennessee Winter (non-hardy variety)	97	90 (Field-grown)
Kearnəy (hardy variety)	97	85 (Field-grown)

These results agree with the earlier ones in showing that plants adapted to drough may be incapable of increasing their very low drought tolerance on exposure to moderat drought. As in the case of soybean, they appear to undergo a "pseudohardening"—i.e an increase in drought avoidance. Thus, we can now understand the paradox tha many drought resistant plants are not frost resistant, yet drought tolerance and fros tolerance are correlated. It is because drought resistance is so commonly due com pletely to avoidance.

Another basic problem is the difficulty in evaluating many of the results of in vestigations on drought resistance. This difficulty arises because the drought re sistance is seldom, if ever, measured. In field experiments, the attempt is made t measure it indirectly by determining yield. Even if this were theoretically acceptabl

the drought is never the same in any two experiments, and therefore the results of two investigators, or of one investigator for two years are not comparable. Furthermore, the property being measured may be an ability to mature before the onset of the severest drought rather than a real ability to survive drought (Chinoy). It is also conceivable that a reduced yield may be more apparent than real, since the below ground growth is completely ignored (Simonis 1952). It is therefore, obvious that little advance in our understanding of drought resistance can be expected until the development of a systematic method of investigating and measuring these different responses of the plant to drought. The following steps are, therefore, suggested:

(1) The actual drought resistance should be measured. If there is no demonstrable difference between the resistance of two varieties, one may yet yield more than the other but this would be due to other causes (see Table I) and the problem would no longer be one of drought resistance. If a difference in drought resistance can be demonstrated, the next step is followed.

(2) The two components—drought avoidance and drought tolerance—should be separately measured in order to determine how much of the overall resistance is due to one or the other.

(3) If avoidance is responsible for the resistance—transpiration, absorption, and translocation of water must be measured, to determine which of these is (or are) responsible for the avoidance. If tolerance is responsible for the resistance—cell sap concentration, bound water, etc., should be measured. It is conceivable that, in some cases, both avoidance and tolerance might be involved and both kinds of measurements would have to be made.

(4) The mechanisms by means of which the plant is able to produce these changes (e.g. reduced transpiration, increased cell sap concentration, etc.) must then be investigated.

The above plan is still beyond the reach of the present-day investigator because the methods for some of the steps have not, as yet, been developed. The plan is, in fact, stopped at the very first step, for lack of a quantitative method of measuring the total drought resistance of a plant. It is largely because of this lack that research in drought resistance has lagged behind that in other fields. This is in sharp contrast to frost and heat resistance, which are both easily measured by determining, respectively, the freezing temperature and the high temperature capable of killing 50% of the plant. There are two good reasons for this lack of a method: a) Because the drought (or relative humidity) is much more difficult to control than temperature. There have been attempts to determine drought resistance in drought chambers, but they have not been too successful (see Clark and Levitt 1956), partly because of this difficulty. b) Because avoidance is at least as important as tolerance in the case of drought resistance, but is of little or no importance in the case of frost and heat resistance. If it were not for this fact a measurement of drought tolerance would be sufficient, and this is readily obtained (see above).

As in the case of frost and heat resistance, the basic definition for the drought resistance of a plant is the degree of drought that results in 50% killing. But how is this degree of drought to be measured? Unlike frost, but like heat, the capacity factor—i.e. the time during which the drought is maintained—is just as important as the intensity factor. Resistance can, therefore, be measured either by keeping the intensity constant and varying the time, or by keeping the time constant and varying the intensity. In practice, the former is much more easily obtained, since it is not easy to vary relative humidity by small but constantly maintained steps. In the method used below, drought resistance is, therefore, defined as the time at 15% R.H. and 30°C that produces 50% killing. This relative humidity was chosen because it is the lowest that can be maintained constant by the method described. It will, therefore, produce results in a minimum of time and can be expected to kill all drought sensitive tissues if maintained long enough.

The above definition for drought resistance can serve as the basis for a practical and simple method of measuring the quantity. But there remains a difficulty inherent in the complexity of drought resistance. In order to measure total drought resistance, all the factors must be included: (1) resistance to loss of water, (2) enhanced water uptake, and (3) tolerance of a decrease in water content. But the simplest way to apply the method is by use of the shoot severed from its roots. The drought resistance of the shoot includes factors (1) and (3) but excludes (2). In order to include (2) the roots must be left on the plant and the water environment of the roots must be controlled. Such control is not possible in soil. It is possible with solutions, provided that the solute is excluded from the root system. By controlling the concentration of the solution, the free energy of the water may be kept under precise control. This provides certain problems that will be discussed in another publication. The present discussion will be confined to the application of the method to shoots without their roots.

The drought chamber is shown in Figure 1. The relative humidity gauge proves that the relative humidity of the air as it leaves the chamber is still about 15%. As

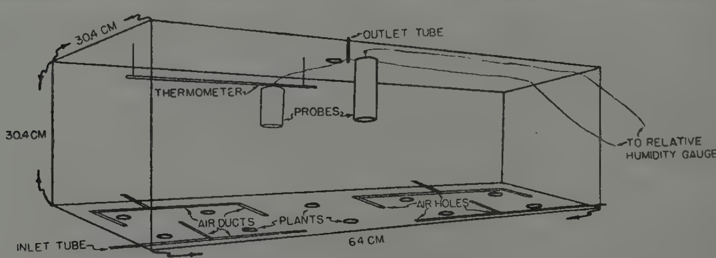

Figure 1
Plexiglass drought chamber with front panel removed in preparation for setting shoots in the ten holes by means of split rubber stoppers.

water is removed from the incoming air by the saturated LiCl solution, the excess salt goes into solution. It is, therefore, necessary to replace the solution every day or so (depending on the relative humidity of the air in the room) by a second erlenmeyer of saturated LiCl. The first one is then boiled until sufficient excess salt separates.

The small chamber illustrated accommodates 10 plants, though any convenient size may be built. At predetermined intervals, two seedlings are removed and their bases cut under water to remove air blocks. They are then left in water to permit recovery, and 24 hours later the amount of injury is estimated. In this way, the time yielding 50% injury can be determined. The drought resistance of the several species of plants tested so far ranges from 6 hours to about a month (Table IV). Since the chamber is small, inexpensive, and easy to construct, it should prove of great value in any attempt to understand the drought resistance of plants.

TABLE IV

Drought resistance of shoots of different plants at 15% R.H. and 30°C, under continuous light 4-40W fluorescent lamps at 2 ft.

Species	Time for 50% killing
Oxalis sp.	5–6 hrs.
Lycopersicon esculentum (tomato)	11 hrs.
Mentha (*citrata* ?)	23 hrs.
Hordeum vulgare (barley)	24 hrs.
Helianthus annuus (sunflower)	31–34 hrs.
Brassica oleracea (cabbage)	48–53 hrs.
Setcreasea striata	32 days

Acknowledgement

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REFERENCES

Chinoy, J. J., Personal communication.

Clark, J. A. and Levitt, J., 1956, The basis of drought resistance in the soybean plant, *Physiol. Plant.*, **9**, 598–606.

Oppenheimer, H. R., 1959, Adaptation to drought: Xerophytism. UNESCO/ES/AZ/415, Paris, 31 March, 1959.

Simonis, W., 1952, Untersuchungen zum Dürreeffekt. I. Morphologische Struktur, Wasserhaushalt, Atmung und Photosynthese feucht und trocken gezogener Pflanzen, *Planta* **40**, 313–332.

Sullivan, C. Y. and Levitt, J., 1959, Drought tolerance and avoidance in two species of oak *Physiol. Plant.*, **12**, 299–305.

QUANTITATIVE EFFECTS OF INTRASPECIFIC COMPETITION: AN EXPERIMENT WITH MANGOLDS

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ABSTRACT

In a field trial with mangolds it was found that the effects of individual competitors spaced evenly around an experimental plant on the logarithm of its root weight were additive provided the number of competitors did not exceed six.

The effect of a given number of competitors placed at different distances x on root weight w could equally satisfactorily be expressed by an equation of the Mitscherlich type:

$$\log (A - w) = \log A + Bx$$

or by a "power equation":

$$\log w = A + B \log x$$

The "reciprocal equation" based on a logistic growth curve gave rather less satisfactory agreement.

INTRODUCTION

Quantitative effects of competition on plant growth have usually been approached empirically, and from the point of view of the effect of varying density of population. Earlier work on the subject was reviewed by Clements, Weaver and Hanson (1929). Literally thousands of experiments on the subject with different crop plants have been performed; but the experimenter has usually been content simply to find what plant population (and perhaps arrangement) has under the particular conditions of the experiment given the highest yield per unit area. Sometimes (e.g. Crowther *et al.* 1937) interaction between plant population and some other factor, such as fertilizer or irrigation regime, have been investigated. But the attempts to reduce results to simple or generalizable laws have been few.

Mitscherlich (1919) attempted to extend his well-known formulation of fertilizer effects to cover effects of plant population. In this, yield is given by

$$y = Y(1 - e^{-cx})$$

where x is the level of the operative factor and Y and c are constants. Mitscherlich claimed that this equation described the relationship both of yield per unit area to population density and of yield per plant to mean available space (i.e. the reciprocal of population density). Other investigators, however (e.g. Redecker 1930, Donald

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1951, Kira *et al.* 1954), were not able consistently to fit this expression to experimenta data.

Warne (1952, 1953a,1953b) used the expression

$$w = Kd^a$$

to describe the relationship between crop weight per unit area (w) and populatior density (d), K and a being constants. The same expression was used independentl by Kira and his collaborators (1953, 1954, 1956); but they subsequently (Shinozak and Kira 1956) regarded it as an approximation to a more precise formulation

$$1/w = Ad + B$$

derived from the assumptions that the growth curve was logistic, and that yielc per unit area became independent of density at high densities.

Sakai and Suzuki (1954a, 1954b, see also Sakai 1955) claimed on the other hanc that their data for barley showed a relation of individual plant yield to spacin expressed by the equation

$$y = A - Bz$$

where y represents the proportional difference in yield of a variety in mixed anc in pure stands with the same spacing, and z is $\log_{10}$ ($\log_2$ spacing).

The relationship between yield and plant population has perhaps been obscurec rather than clarified by the common practice of expressing yield in terms of unit area This has the effect of introducing the independent variable again in the form o expression of the dependent variable, which can be avoided if yields are expressed pe plant. Considering each plant as an individual, whose yield is affected by the plant around it, has the additional advantage of making it easier to treat the effects o different plant arrangements concurrently with those of different population densities

If one takes the yield of a plant as affected by the presence of a single competin plant in its vicinity, and one assumes that the species and size of the latter are fixed the effect may be taken to depend on the distance separating the two plants. Wha form is this relationship likely to take? In the first place, one may say that it i reasonable to express the effect on yield in logarithmic rather that absolute terms in view of the approximate constancy of the relative growth rate during a substantia portion of the life of the plant and the expectation that competitive effects woulc shew themselves in a reduction in relative growth rate (Donald 1951, Koyama anc Kira 1956, Yoda *et al.* 1957).

Competition above ground will take effect through a reduction in light intensit on the foliage. The proportion of the sky obscured by a competitor of fixed siz will be nearly inversely proportional to the square of its distance; and the vertica component of the radiation intercepted will thus be, on average, roughly inversel proportional to the cube of the distance. To a first approximation, then, the radiatior normal to a leaf placed horizontally at ground level will be reduced by an amoun inversely proportional to the cube of the distance of a competitor. In view of the

well-known general form of the curve connecting photosynthesis with light intensity — which in the field commonly approaches saturation — this means that the reduction in photosynthesis through competitive reduction in light intensity will over a considerable range increase roughly in proportion to the inverse cube of the distance. The effect on relative growth rate is somewhat alleviated by a favourable effect on leaf area ratio (Blackman and Wilson 1951).

If one now considers competition between the underground organs, data on the concentration of root absorbing surface in zones at different horizontal distances from the stem base are rather scanty; Evans' (1937) observations on sugar-cane suggest that it falls off approximately linearly with distance, so it may be assumed that the intensity of competition for water and nutrients will follow the same rule. And since growth responses to differences in the supply of water and nutrients, like those to light intensity, follow a law of diminishing returns, the effects of below-ground competition on yield should increase with proximity rather more than in inverse proportion to the distance.

The relation of competitive effects to distance is thus likely to differ considerably according to whether competition for water and mineral nutrients, or for light, predominates. The effect should increase inversely as a power of the distance ranging between the first and the third power, according to the relative importance of below-ground and above-ground competition. If the power in question is the square, yield per unit area of a regular planting becomes independent of density — the limiting condition postulated by the Osaka workers.

Admittedly the discussion above can be no more than a first approximation, since the competitor is treated as a constant element of the environment, whereas in most situations the intensity of competition is changing throughout the plants development. Furthermore competition is reciprocal, and the effects on the growth of the competitor itself have not been taken into consideration.

When (as is usual) there are several plants competing with a single individual, the question arises as to the way in which their separate effects are combined — are they in any sense additive? For Sakai (1955, 1956, 1957) they are — at any rate, the differences in competitive effects between two varieties are additive. No one else appears to have studied the question.

The experiments described in the present paper were intended to shed a little more light on these two questions — what function relates the competitive effect of one plant on another (expressed as a decrease in the logarithm of the weight of the plant or its organs) to the distance separating them? and to what extent and in what sense are the competitive effects of different individuals additive?

EXPERIMENTAL WORK AND RESULTS

To elucidate the two questions posed in the introduction, experiments were set up in the two years 1955 and 1956, in the garden of the Agricultural Botany Department of Reading University at Shinfield. A yellow globe variety, Sutton's Prizewinner,

of mangold (*Beta vulgaris*) was chosen as the material for the experiment. It was necessary to compare the yield of each plant subjected to competition as accurately as possible with that of a control plant free from competition, so the unit plot consisted of two plants of which the yield was observed — one experimental and one control. The plot measured 3.2×1.6 metres, and thus each plant measured was in the centre of a sub-plot 1.6 metres square. The experimental sub-plot included different numbers of other plants at different distances from the centre; but in no case were these closer than 1.1 metres from the control plant, at which distance it was assumed that effects of competition would be negligible.

The results of the experiment of 1955 were attended by error components too large for them to be usefully informative. But the results of 1956 were much more clear-cut, and this experiment is accordingly reported in detail.

The experiment was set up in five randomized blocks, each consisting of 32 plots of the size mentioned. The competition imposed was all of the same variety of mangold, but sown two weeks before the plants to be recorded. The competing plants were sown on May 1 and 2, 1956, at eight different distances from the experimental plant (10, 13, 16, 20, 25, 30, 40, and 50 cm); these spacings may be compared with normal practice of about 60×30 cm for mangold crops in England. One, two, four or eight competitors were established, arranged equally around the experimental plant, their azimuth being determined at random. Experimental and control plants were sown on May 15, 1956, and each site, both for these plants and the competers, was thinned to a single seedling three weeks later. All weed competition was carefully eliminated throughout the growing season. The roots were harvested and weighed between November 14 and 16, 1956. The mean root weight for the control plants was 2012 grams.

The mean competitive effect (difference in log. weight of control and experimental roots) for each treatment combination is shewn in Table I, and the analysis of variance in Table II.

TABLE I

Mean decrease in log (root fresh weight) as a result of competition

Distance (cm)	Number of competitors 1	2	4	8	Mean
10	0.58	1.24	2.32	2.24	1.59
13	0.58	0.59	2.00	2.35	1.38
16	0.51	0.78	2.35	2.60	1.56
20	0.16	0.55	1.51	2.66	1.22
25	0.68	0.33	1.61	2.74	1.34
30	0.14	0.95	1.67	2.56	1.33
40	0.07	0.48	1.26	1.55	0.84
50	0.18	0.70	0.37	0.83	0.52
Mean	0.36	0.70	1.64	2.19	1.22

TABLE II
Analysis of variance for decrease in log (root fresh weight)

	Degrees of freedom	Sum of squares	Mean square	F	P
Blocks	4	1.905	0.4762	—	—
Number of competitors	3	84.575	28.1917	54.05	$<.001$
Distance	7	18.925	2.7035	5.18	$<.001$
Number x distance	21	16.192	0.7711	1.48	$>.05$
Error	121	63.112	0.5216		

DISCUSSION

Let us consider first the effect of different numbers of competing plants. It will be seen immediately from the data in Table I that the mean effect of two plants is very close to double the effect on one competitor, and that the mean effect of four is little more than double that of two. So far, then, the hypothesis of additive effects is upheld. But eight competitors do not have double the effect of four. On consideration, this seems reasonable, since the sides of an octagon are shorter than the radius of the circle in which it is inscribed, and consequently eight competitors will influence one another's growth more than the growth of the experimental plant. The maximum effect of a circle of competitors might be expected to be reached when their number is six; this seems in fact to have been the assumption of Sakai and Suzuki (1955a, 1955b; see also Sakai 1957) in their studies of competition. If now we test this new hypothesis — that the effect of competitors equally spaced in a circle around the experimental plants is additive up to six, but the additional plants beyond this number have no additional effect — against the treatment means the agreement is excellent (Figure 1), the residual variance between plots with different numbers of competitors not being significant.

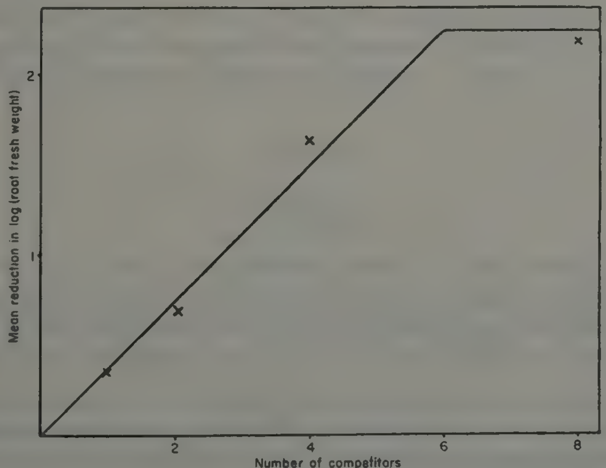


Figure 1
Competitive effect of different numbers of individuals (x) on log root weight (y).

Reference was made in the Introduction to Sakai's (1955, 1956, 1957) conclusion that the effect of varying numbers of plants of a different variety (in a circle of six competitors around the plant under observation) was linearly additive. This conclusion of his, however, applied to the yields as measured rather than to their logarithms. But the differences were relatively small, and if competitive effects on the logarithm of yield are computed it is found that their relationship to numbers of competitors was at least as close to linear as that of the yields themselves; this is shewn by the correlation coefficients represented in Table III.

TABLE III

Effects of different numbers of "competitors" (from Sakai 1957)

Coefficient of correlation with number of "competitors"

	Yield	Log yield
Barley (systematic arrangement) : ear weight	—.900	—.890
Barley (random arrangement) : ear weight	—.931	—.937
Upland rice : kernel weight	—.949	—.970
Red rice : kernel weight	+.921	+.930

Sakai's results are thus in very satisfactory agreement with those reported here.

The hypothesis of additive effects has been tested further on the data of Wiggans (1939). If it is true, then yield of a row crop should be represented by

$$\log w = a + b_1 \log x_1 + b_2 \log x_2$$

where w is the weight per plant, and x_1 and x_2 are the spacing within the row and between rows respectively. If this regression is computed from Wiggans' data, a very satisfactory fit is obtained (multiple correlation coefficient=0.9926) with the regression coefficients

$$b_1 = 0.853$$

$$b_2 = 0.757$$

differing significantly. In other words, the competitive effect of a row is indeed additive with that of adjacent plants in the same row, but less than that of a single plant at the same distance (instead of more marked as would be true if each plant in the adjacent row exercised an effect inversely proportional to the square of its distance).

This implies that the competitive effect of a plant in an adjacent row is reduced — perhaps through inter-row cultivations — and that different rows behave to some extent independently (c.f. Kira *et al.* 1956).

As regards the effect of distance the overall results shew a general decrease in competitive effect as spacing increases; but, surprisingly, this decrease is significant only from 30 cm onwards. Over the whole range of data, the interaction term does not quite reach significance. As between the three widest spacings, however, the interaction does attain significance, as is to be expected if the effects of individual competitors differ with spacing and are additive.

If we now proceed to compare the spacing effects observed with those expected on the various hypotheses mentioned in the introduction, we find first that we must discard the formulation of Sakai and Suzuki (1954a, 1954b; see also Sakai 1955) — which is in any case rather unacceptable because the form of the curve connecting competitive effect with $\log_{10}$ ($\log_2$ spacing) is bound to depend on the unit of measurement, as well as on the bases of the logarithms. Working with the logarithms used by Sakai, and a spacing unit of 5 cm (which, as in his case, puts the independent variable for the closest spacing used at zero), the results shown in Figure 2 are obtained. The relationship is clearly very far from linear.

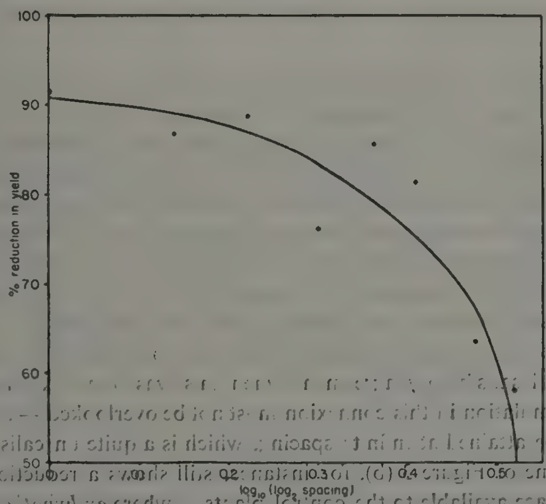


Figure 2

Percentage reduction in root weight (y) through two competitors, related to $\log_{10}$ ($\log_2$ spacing) (x).

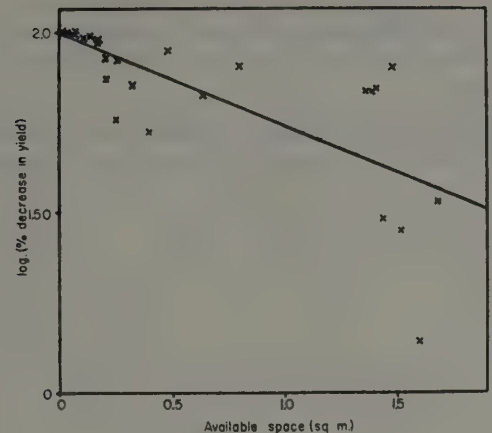
Other formulations are designed for regular stands, the independent variable being density or available space per plant rather than a linear measure of spacing. The inadequacy of such formulations which ignore possible effects of plant arrangement is shewn by data such as those of Wiggans (1939) with soybean. He obtained

the same population densities by various combinations of spacing within and between rows, and found that yields increased as spacing within and between rows became more similar — in other words, as the pattern approached a square planting. In the data of Garner and Sanders (1939) with sugar beet an arrangement 18″ × 12″ was found to give higher yields than 24″ × 9″, with the same population density. Similar results emerge from the tables of Warne (1951) for globe beet, where almost identical plant populations gave slightly higher yields where they were obtained by closer rows with a wider thinning distance; with maize, too, Mooers' (1920) results led to a preference for the widest practicable spacing within the row. Hodgson and Blackman (1956), on the other hand, found no effects of spatial arrangement on the yield of broad bean at a given population density. A square planting arrangement as optimum would accord with the hypothesis of additive effects proposed in the present paper.

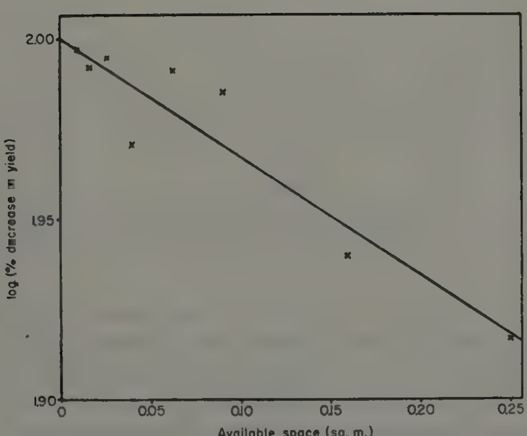
For the purpose of comparing the mangold data with formulations based on plant density (or available space per plant) rather than inter-plant distance, the space available was taken as limited by the perpendicular bisector of the lines joining the experimental plant to each competitor, and by the boundaries of the sub-plot in other directions.

Using this measure of available space, the Mitscherlich formulation gives the results shown in Figure 3 (a). Agreement is reasonably satisfactory, though the concentration of points at high density and low yield makes it difficult to judge the goodness of fit. The additive character of individual competitive effects makes it permissible to derive a combined estimate of the effect of a single competitor from each group of four treatments at a common spacing distance. The expected effects with square planting have been computed on this basis and are shown on a different scale in Figure 3 (b); the agreement with linearity is still good. The regression lines in these diagrams have been fitted using the observed root weight of the control plants as the value for the maximum yield, as was done by Kira *et al.* (1954) — though they did not find satisfactory agreement when this was done. A drawback of the Mitscherlich formulation in this connexion must not be overlooked — that maximum yield can only be attained at infinite spacing, which is a quite unrealistic condition; the regression line of Figure 3 (b), for instance, still shows a reduction in yield of 22.7% at the space available to the control plants — where *ex hypothesi* competitive effects are nonexistent.

The expression used by Warne, and by the Osaka workers in their earlier publications, also gives satisfactory general agreement with these data as is shown in Figure 4. The line cuts the × -axis almost exactly at the space available to the control plants. This expression has, like that of Mitscherlich, only limited applicability, for it can hardly be expected to cover low densities with competition virtually nonexistent. However, within the range covered in this experiment the effects of competition seem to be adequately described by this equation.



a



b

Figures 3(a) and 3(b)

The Mitscherlich formulation as an expression of the effect of competition. The logarithm of the reduction in yield (y) is related to the available space (x). In Figure 3(a) the points represent the mean for each combination of number and distance of competitors; in Figure 3(b) a general mean for each spacing distance has been derived.

Despite the better theoretical basis for the 'reciprocal equation' of the Osak workers, agreement is less satisfactory, as may be seen from Figure 5. At low densities the points seem to depart markedly from a linear relationship, and at high densitie they are very scattered.

In the approximative 'power equation' of Figure 4, it is interesting to note tha the regression coefficient a is 1.065—very close to the value of unity which is claime

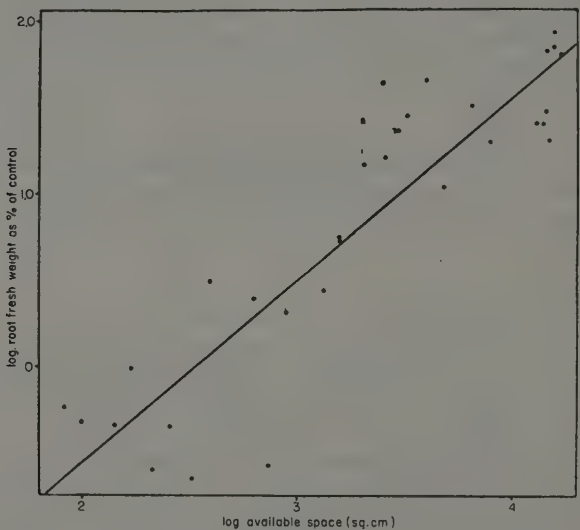


Figure 4
Relationship between log. root weight (y) and log. available space (x).

by Kira and his collaborators as standard at higher densities ('the law of constan final yield'). This standard value, however, was regarded as applying to the dr weight of the whole plant, and the Osaka workers expected the weight of individua organs to be related allometrically to plant dry weight, and hence to have a valu of a differing from unity (Kira *et al.* 1953, 1956; Shinozaki and Kira 1956).

We find then, that a 'power equation' gives a good empirical fit to the observations and that the influence of a competitor is nearly inversely proportional to the square of the spacing distance — a situation appropriately intermediate between the inverse proportionality to the cube of the distance expected in competition for light, and the inverse proportionality to the distance itself expected for root competition.

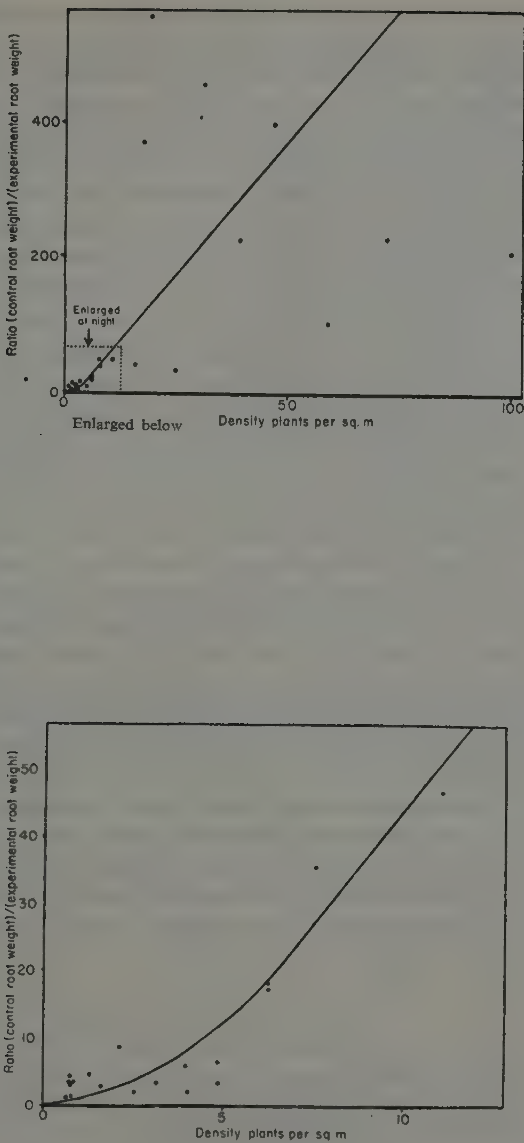


Figure 5

Relationship between the reciprocal of root weight (y) and the density of population (x). The graph below is an enlargement of the part of the upper graph near the origin.

Kira and his collaborators checked their 'power equation' and 'reciprocal equation' against various sets of data in the literature. In connexion with the present work, a number of other sets of data were tested. Engledow and his collaborators (1928), for instance, published data for sugar beet with different numbers of plants in unit length of row; agreement with both equations, particularly the 'reciprocal equation', was good. The data of Sakai and Suzuki (1954a, 1954b) also agreed particularly well with the 'reciprocal equation', and rather less satisfactorily with the 'power equation'. Donald (1954) and Hodgson and Blackman (1956) published data agreeing well with both. All these investigators were however investigating *average* effects of population density in regular plantings, rather than the effects of one individual on another.

Despite the satisfactory agreement of the present data with the Mitscherlich 'law', it must be rejected as a general formulation of the effects of spacing in view of the substantial disagreements reported elsewhere in the literature. The 'power equation' has almost always given satisfactory agreement, and is a convenient form, though without any theoretical basis; it also lends itself well to additive treatment of the effects of individual competitors, as shown in respect of Wiggans' data above. It clearly fails at low densities, when the absence of competition must render individual plant weights independent of density; realizing this, Kira *et al.* (1954) postulated a truncation of the curve at the maximum weight reached under unrestricted growth. Such a truncated curve is aesthetically unsatisfying, and their preferred 'reciprocal equation' gave a curve asymptotic to this maximum weight (which, however, does not avoid the objection raised against the similarly asymptotic Mitscherlich curve). The 'reciprocal equation', based as it is on the reasonable assumption of a logistic curve of growth, is very attractive; like the 'power equation' it lends itself to expressing additive effects of different competitors (c.f. Maskell's 'resistance formula' - Balmukand 1928). So far the 'reciprocal equation' has met most of the demands imposed upon it; and although the agreement shewn in Figure 5 seems unsatisfactory, this should not lead one so far as to condemn the formulation. What is required is a rigorous technique for fitting the formula to experimental data, and for testing goodness of fit. Until the applicability of the expression can be fully examined in this way, it may be accepted with reserve. Meanwhile the 'power equation' provides a very satisfactory approximation.

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REFERENCES

BALMUKAND, B., 1928, Studies in crop variation. V. The relation between yield and soil nutrients, *J. agric. Sci.*, **18**, 602–627.

BLACKMAN, G. E. AND WILSON, G. L., 1951, Physiological and ecological studies in the analysis of plant environment. VII. An analysis of the differential effects of light intensity on the net assimilation rate, leaf-area ratio, and relative growth rate of different species, *Ann. Bot., Lond.*, N.S. **15**, 373–408.

CLEMENTS, F. E., WEAVER, J. E. AND HANSON, H. C., 1929, *Plant Competition*, Carnegie Institution of Washington.

CROWTHER, F., TOMFORDE, A. AND MAHMOUD, A., 1937, Experiments in Egypt on the interaction of factors in crop growth. 8. Manuring of cotton in Egypt. An account of the work of the Joint Agricultural Research Scheme, *Bull. tech. Sect. Soc. Agric. Cairo*, **32**, 1–38.

DONALD, C. M., 1951, Competition among pasture plants. I. Intraspecific competition among annual pasture plants, *Aust. J. agric. Sci.*, **2**, 355–376.

DONALD, C. M., 1954, Competition among pasture plants. II. The influence of density on flowering and seed production in annual pasture plants, *Aust. J. agric. Res.*, **5**, 585–597.

ENGLEDOW, F. L., MAHER, C. A., SMITH, J. H., WILLIAMS, H. R., FAIL, H. AND RAYNS, F., 1928, Yield and plant population in sugar beet, *J. agric. Sci.*, **18**, 574–601.

EVANS, H., 1937, Further investigations on the root-system of sugarcane, *Bull. Mauritius Sugar Res. Sta.*, **12**, 1–33.

HODGSON, G. L. AND BLACKMAN, G. E., 1956, An analysis of the influence of plant density on the growth of *Vicia faba*. I. The influence of density on the pattern of development, *J. exp. Bot.*, **7**, 147–165.

KIRA, T., OGAWA, H. AND SAKAZAKI, N., 1953, Intraspecific competition among higher plants. I. Competition–yield–density interrelationship in regularly dispersed populations, *J. Inst. Polytech. Osaka*, **D4**, 1–16.

KIRA, T., OGAWA, H. AND HOZUMI, K., 1954, Intraspecific competition among higher plants. II. Further discussions on Mitscherlich's law, *J. Inst. Polytech. Osaka*, **D5**, 1–17.

KIRA, T., OGAWA, H., HOZUMI, K., KOYAMA, H. AND YODA, K., 1956, Intraspecific competition among higher plants. V. Supplementary notes on the C–D effect, *J. Inst. Polytech. Osaka*, **D7**, 1–14.

KOYAMA, H. AND KIRA, T., 1956, Intraspecific competition among higher plants. VIII. Frequency distribution of individual plant weight as affected by the interaction between plants, *J. Inst. Polytech. Osaka*, **D7**, 73–94.

MITSCHERLICH, E. A., 1919, Ein Beitrag zur Standraumweite unserer landwirtschaftlichen Kulturpflanzen in Gefässen und im freien Lande bei Reinsaat und Mengsaat, *Landw. Jb.*, **53**, 341–360.

MOOERS, C. A., 1920, Planting rates and spacing for corn under southern conditions, *J. Amer. Soc. Agron.*, **12**, 1–22.

REDECKER, W., 1930, Der Einfluss der Standweite der Gemüsepflanzen auf den Pflanzenertrag, *Gartenbauwiss.*, **3**, 395–421 (Cited by Kira *et al.*, 1953).

SAKAI, K. I., 1955, Competition in plants and its relation to selection, *Cold Spring Harbor Symp.*, **20**, 137–157.

SAKAI, K. I., 1956, Further note on the effect on competition of a varying number of competing and non-competing individuals, *Rep. nat. Inst. Genet. Japan*, **6** (for 1955), 79–80.

SAKAI, K. I., 1957, Studies on competition in plants. VII. Effect on competition of a varying number of competing and non-competing individuals, *J. Genet.*, **55**, 227–234.

SAKAI, K. I. AND SUZUKI, Y., 1954a, Studies on competition in plants. III. Competition and spacing in one dimension, *Jap. J. Genet.*, **29**, 197–201.

SAKAI, K. I. AND SUZUKI, Y., 1954b, Competition and spacing in one dimension in plants, *Rep. nat. Inst. Genet. Japan*, **4** (for 1953).

Sakai, K. I. and Suzuki, Y., 1955a, Studies on competition in plants, II. Competition betwee diploid and autotetraploid plants of barley, *J. Genet.*, **53**, 11–20.

Sakai, K. I. and Suzuki, Y., 1955b, Studies on competition in plants. V. Competition betwee allopolyploids and their diploid parents, *J. Genet.*, **53**, 585–590.

Shinozaki, K. and Kira, T., 1956, Intraspecific competition among higher plants. VII. Logist theory of the C–D effect, *J. Inst. Polytech. Osaka*, **D7**, 35–72.

Warne, L. G. G., 1951, Spacing experiments on vegetables. VI. A comparison of the results ob tained in 1948 and 1949 in experiments on thinning with root crops in Cheshire, with suggestion as to desirable plant populations for these crops, *J. hort. Sci.*, **27**, 110–116.

Warne, L. G. G. 1953a, Spacing experiments on vegetables. VII. The growth and yield of glob beet, parsnips and carrots grown at several spacings in two adjacent fields of different fertility *J. hort. Sci.*, **28**, 114–120.

Warne, L. G. G., 1953b, Spacing experiments on vegetables. VIII. The responses of several varietie of globe beet, long beet and parsnips to changes in the thinning distance, *J. hort. Sci.*, **28**, 152–15

Wiggans, R. G., 1939, The influence of space and arrangement on the production of soybean plant *J. Amer. Soc. Agron.*, **31**, 314–321.

Yoda, K., Kira, T. and Hozumi, K., 1957, Intraspecific competition among higher plants. IX Further analysis of the competitive interaction between adjacent individuals, *J. Inst. Polytech Osaka*, **D8**, 161–178.

CONTRIBUTION TO THE KNOWLEDGE OF THE ECOLOGY OF THE "RIO NEGRO CAATINGA" (AMAZON)

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ABSTRACT

The present paper gives results obtained in Taraquá (0°4'N 68°14'W alt. 100 m). The vegetation studied is known as "Rio Negro Caatinga", an open type of evergreen forest which occurs in the upper Rio Negro amidst the exuberant Amazonian rain-forests.

The yearly rainfall in the region may be more than 4,000 mm and there is hardly a month with less than 100 mm. Average temperatures and relative humidities are high. Soils are in general very poor.

Detailed information on the vegetation from taxonomical, sociological and anatomical viewpoints, will be presented elsewhere.

The present paper gives data on: degree of stomatic openings of 27 species from 8.30 a.m. to 5.00 p.m., daily march of transpiration, velocity of stomatic movements and saturation deficits of several species.

The main results are: 1) no restriction in transpiration was observed in general; 2) when some restriction occurred, this was followed by a decrease in the size of stomatic openings, 3) movements of stomates are in general slow, 4) cuticular transpiration is high, 5) saturation deficits were not in general more than 5% of the maximum water content.

Conclusions: 1) water cannot be the limiting factor of the vegetation studied, 2) anatomical features and water relations of this vegetation are very similar to those found in the Brazilian "cerrados", 3) the "Rio Negro Caatinga", similarly to the "cerrado"-vegetation, seemed to be conditioned by soil deficiencies.

INTRODUCTION

Vhen talking about the Amazon, one always thinks of the jungle. However, it hould be remembered that amidst the exuberant Amazonian rain forests one finds, n many places, islands of different types of vegetation, such as "cerrados"* and 'caatingas"**.

Whereas the Amazonian "cerrados" are similar to the "cerrados" of central and outhern Brazil, the Amazonian "caatingas" have nothing in common with the ortheastern "caatingas".

In the Brazilian Northeast the yearly rainfall is about 500 mm and there is a dry eriod of 8–9 months; relative humidity is in general very low and since temperatures

* "Cerrado" is a Brazilian word for a very widespread savannah-like vegetation.

** "Caatinga" is an Indian word meaning clear forest.

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are high, evaporation is also high. These conditions gave rise to the developmen of a special vegetation quite well adapted to survive long periods of severe drought Among other adaptations there is a general drop of leaves.

In the Amazonian "caatinga" of the upper Rio Negro the yearly rainfall ma be as much as 4,000 mm without a dry period. The vegetation is evergreen.

In the Northeast, throughout the dry period, there is a leafless, clear vegetation In the Amazonian caatinga, though evergreen, the vegetation is also clear becaus it is quite open and contrasts with the dark rain forest nearby.

The ecologist finds here an interesting problem: Why, under the same genera climatic conditions and side by side with the magnificent rain forest, does ther appear such a poor type of vegetation?

The abundance of rainfall and lack of dry season excludes moisture as a limitin factor. But this is not so simple. The soil is almost pure sand, and thus its wate retaining power must be very low. In this way a few rainless days may bring abou a more or less pronounced drying of the superficial layers where most of the root are found, and the vegetation may suffer from this dry condition.

These are the problems the present paper will deal with.

The experimental work was performed in Taraquá (Rio Negro), close to th border between Brazil, Columbia and Venezuela, where we stayed approximatel a month.

2. CLIMATIC AND EDAPHIC CONDITIONS

Taraquá lies at 0°4′N, 68°14′ W and the altitude at the meteorological statio is 100 m above sea-level.

The yearly rainfall can be greater than 4,000 mm. There is hardly a month wit less than 100 mm.

In the absence of good meteorological records in Taraquá we will give som data from the station of Uaupés (formerly São Gabriel), which can be taken a representative of the region. Uaupés lies at 0°8′ S, 67°5′ W and has an altitud of 84.14 m at the meteorological station. The average of minimal temperatures i more than 20°C, of maximal temperatures more than 30°C and of relative humiditie more than 88%. The average yearly rainfall is about 3,000 mm, October being th driest month, with an average precipitation of 160 mm.

Information on the daily march of evaporation in Taraquá, in the days whe our work was performed, will be presented with the experimental results.

As we said before, the soil in Taraquá, in which the caatinga vegetation grows is in general almost pure sand with sometimes, in spots, a thin superficial laye of organic matter.

Detailed analysis performed by Lúcio S. Vieira of the Instituto Agronômico d Norte, Belém, Pará will be published later.

3. THE VEGETATION

A detailed study of the caatinga vegetation in Taraquá from the taxonomic and sociological viewpoint will be published by João Murça Pires, Instituto Agronômico do Nortem, Belém, Pará.

In the present paper, physiological data will be given on 27 species selected from among the most frequent ones. Our selection includes plants from different families and from different biological groups: herbs, shrubs and trees, climbers, epiphytes, etc.

It is surprising to find in this vegetation, under conditions of high humidity, a great number of sclerophyllous plants. Anatomical studies of leaves especially concerned with the stomates will be published elsewhere by the present author in collaboration with Berta Lange de Morretes, Departamento de Botânica, Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo.

4. WATER RELATIONS

a. Methods

Transpiration measurements were made by the method of rapid weighings with a small torsion-balance (Jung, Heidelberg). Details of the technique employed can be found in previous publications (Ferri 1955, Rawitscher et al. 1943). All values were computed on a leaf surface basis (100 cm^2).

The method used to establish the type of curve representing stomatic reactions in leaves without the regular water supply, was as follows: The leaf was severed from the plant and placed to hang from one arm of the balance. Its initial weight was established within a few seconds. Further measurements were made after longer intervals. This process lasted for different lengths of time, according to the plant studied. The difference between two successive weighings divided by the time elapsed, gave the rate of water loss during that interval.

The determination of stomatic openings was made by Molisch's (1912) method of infiltration with xylol. An arbitrary scale from 0–4 was used, the first value representing complete closure of the stomates and the last the maximal degree of opening. Each time several infiltrations were made for each plant. This was repeated throughout the day during several days. All results were then averaged and put in a final table.

Saturation deficits of leaves were determined according to Stocker's formula (1929).

Meteorological data were obtained with the classical equipment. Evaporation measurements were made with a Piche-evaporimeter, with a disc of green blotting paper of known surface. All values were computed for a unit surface of 100 cm^2.

b. Daily march of transpiration

We studied the daily march of transpiration of 11 species. We found that they can be divided in two main groups: in the first, largest group, we have the species which

do not restrict the water consumption at all or show only a very mild restriction. This restriction is in fact so mild that we are not sure if it exists at all. To this grou belong: *Sphaeradenia amazonica*, *Bactris cuspidata*, *Cunuria crassipes*, *Retiniphyllum truncatum*, *Pagamea coriacea*, *Hevea rigidifolia*, *Clusia spathulaefolia* and *Lissocarpa benthami*. Figure 1 and Figure 2, which present the daily march of transpiration of *Sphaeradenia amazonica* and of *Cunuria crassipes*, can be used as examples of this type of behaviour.

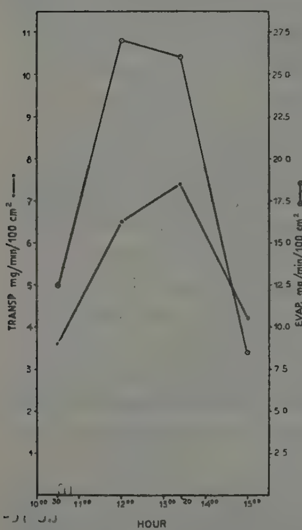

Figure 1

Daily march of transpiration of *Sphaeradenia amazonica* (solid circles) and daily march of evaporation (open circles)

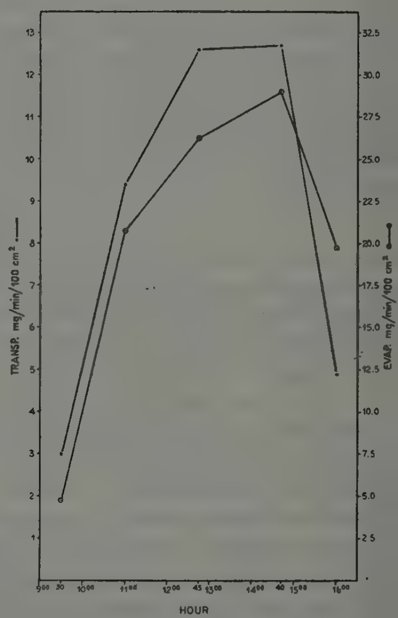

Figure 2

Daily march of transpiration of *Cunuria crassipes* (solid circles) and daily march of evaporation (open circles).

The second group is that of species which show some restriction of transpiration. In this group we have two types of behaviour: some plants, having closed the stomates some time in the morning, probably in response to a weak saturation defici in the morning, will reopen them in the afternoon. These plants will show double peaked transpiration curves. *Compsoneura debilis* and *Zamia lecointei* showed this type. Figure 3 presents the daily march of transpiration of *Zamia lecointei*. The second type appears in plants which after closing the stomates some time in the morning, will not reopen them the same day. The daily curve of transpiration of such

lants will have a single peak which appears some time before the peak of evapora-on. *Maxillaria leucaimata* behaved in this way (Figure 4).

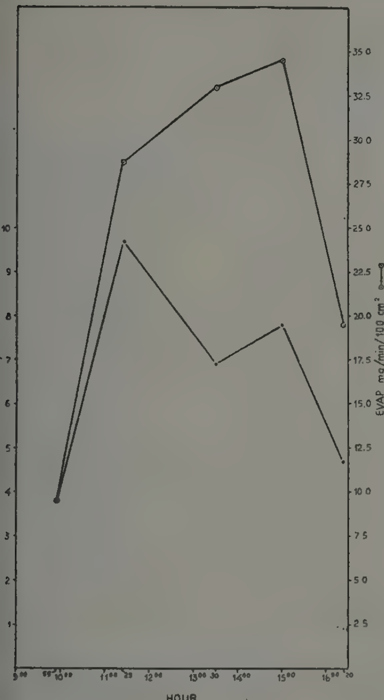

Figure 3

aily march of transpiration of *Zamai lecointei* olid circles) and daily march of evaporation (open circles).

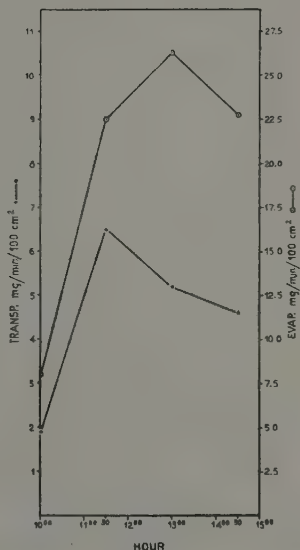

Figure 4

Daily march of transpiration of *Maxillaria leucaimata* (solid circles) and daily march of evaporation (open circles).

It is quite clear that some plants that show a certain type of behaviour under ome conditions, may show a different one if conditions vary. Thus, some plants f *Zamia lecointei* showed a double-peaked transpiration curve, whereas other dividuals of the same species did not reopen the stomates in the same day. Probably ese individuals, for some reason, were not able to restore their turgidity rapidly nough, either due to the fact that they grew on a drier spot of soil or else in a more xposed area.

Figure 5 shows the daily march of transpiration of a plant of this species which fact did not show a double-peaked transpiration curve, in contrast to the plant f the same species which had a behaviour as presented in Figure 3.

c. Degree of opening of stomates throughout the day

Table I groups together all the results of infiltration experiments made in the "R Negro Caatinga" from February 20 to March 2. Each value is the average of at lea three observations.

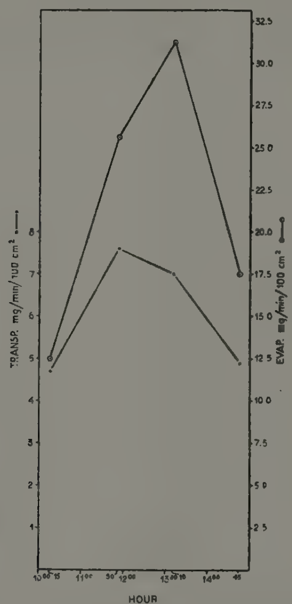

Figure 5

Daily march of transpiration of *Zamia lecointei* (solid circles) and daily march of evaporation (op circles).

We can easily see that most of the species that were studied did not show ar modification at all of the degree of stomatic opening all through the day. In fac *Bactris cuspidata, Bocageopsis multiflora, Catostema sclerophyllum, Clusia spathula folia, Coccoloba excelsa, Epistephium parviflorum, Guatteria insculpta, Hevea rigic folia, Lissocarpa benthami, Macrolobium punctatum* and *Rourea* sp. always ga maximal infiltration with xylol.

Discophora froesii, Haploclathra verticillata, Leretia parviflora and *Sphaeraden amazonica* apparently showed a little less infiltration, either in the beginning, in the middle or else in the end of the day. Such restriction of stomatic openin were so small, however, that we cannot be sure that they really existed.

A second group of plants showed a quite clear decrease in infiltration (which indicative of decrease in size of stomatic openings) some time in the day, with a ne increase later in the afternoon. To this group belong: *Compsoneura debilis, Cunur crassipes, Psamisia leucostoma* and *Zamia lecointei.*

TABLE I

Degree of opening of the stomata as shown by infiltration with xylol
Data obtained from February 20 to March 2, 1959

Species	Time of the day					
	a. m.			p. m.		
	8.30–9.30	10–11	11.30–12.30	13–14	14.30–15.30	16–17
Bactris cuspidata	4	4	4	4	4	—
Bocageopsis multiflora	4	4	4	4	4	4
Catostemma sclerophyllum	4	4	4	4	4	—
Clusia spathulaefolia	4	4	4	4	4	4
Coccolobia excelsa	4	4	4	4	4	4
Compsoneura debilis	3	3	3	2–3	3	3–4
Cunuria crassipes	3	3	2–3	2	2	2–3
Discophora froesii	4	4	4	3	4	4
Epistephium parviflorum	4	4	4	4	4	4
Guatteria insculpta	4	4	4	4	4	4
Haplocathra verticillata	3	4	3–4	4	4	4
Hevea rigidifolia	4	4	4	4	4	4
Leretia parviflora	4	4	4	4	4	3–4
Lissocarpa benthami	4	4	4	4	4	4
Macrolobium punctatum	4	4	4	4	4	4
Maxillaria leucaimata	3	3–4	2–3	1	1	1
Mouriri crassifolia	3	4	4	4	3–4	2–3
Odontadenia sp.	0	2	2	0	0	—
Pagamea coriacea	2–3	4	4	2–3	2	2
Psamisia leucostoma	3–4	4	3	3	1–2	2–3
Rapatea longipes	2	2	2–3	1	0	0
Retiniphyllum truncatum	1	2	1–2	1	0–1	0–1
Rourea sp.	4	4	4	4	4	—
Sobralea sp.	0	0	0	0	0	—
Sphaeradenia amazonica	4	4	4	4	4	3
Tapura guianensis	3	3–4	3–4	2–3	2	2
Zamia lecointei	3–4	3	2–3	0–1	1–2	2

Some species seem to have closed the stomates (at least partially) some time in the day without reopening them, such as *Maxillaria leucaimata*, *Mouriri crassifolia*, *Odontadenia* sp., *Pagamea coriacea*, *Rapatea longipes*, *Retiniphyllum truncatum* and *Tapura guianensis.*

There is, finally, one species among the 27 that were observed, that never showed any infiltration at all: *Sobralea* sp.

If we compare the data obtained with infiltration with the transpiration measurements made by rapid weighings, which appear in the curves representing the daily marchs of transpiration, we will see a quite good agreement. In fact, Figure 1 shows no restriction at all on the daily transpiration of *Sphaeradenia amazonica* and Table I shows always maximal infiltration; Figure 3 is the double-peaked curve of transpiration of *Zamia lecointei* and we can see in the infiltration data that there is a decrease of the degree of infiltration starting between 10 and 11 a.m. and an increase

starting between 2.30 and 3.30 p.m. *Maxillaria leucaimata* (Figure 4) shows the ma-ximum of transpiration at 11.30 and the maximal infiltration occurs approximatel at the same time.

It is clear, then, that every pattern of transpiration is determined primarily by th pattern of stomatic behaviour.

d. Stomatic reactions and cuticular transpiration

Stomatic reactions in leaves taken from the plant and put to hang on the balanc were studied in 11 species: *Clusia spathulaefolia, Lissocarpa benthami, Maxillari leucaimata, Zamia lecointei, Sphaeradenia amazonica, Discophora froesii, Pagame coriacea, Hevea rigidifolia, Epistephium parviflorum, Cunuria crassipes* and *Retini-phyllum truncatum.*

With only small deviations they showed approximately the same general patter of response (Figure 6). The curves representing closure movements of the stomate are not smooth but show rather pronounced fluctuations.

Not a single species showed a very low value of water loss at the end of the experi-ment which lasted sometimes even more than one hour. Some species, after such long period without any water supply, still gave positive values of infiltration wit xylol. Thus, for these plants, apparently, cuticular transpiration is never attained *Zamia lecointei, Discophora froesii, Cunuria crassipes* and *Retiniphyllum truncatu* are examples of such a type of behaviour. Other species, such as *Lissocarpa benthami Maxillaria leucaimata* and *Pagamea coriacea*, did not show infiltration at the en of the experiment, but, nevertheless, showed high values of water loss.

If there is no infiltration with xylol we admit that the stomates are closed (o almost so) and take the water loss value as fairly representing cuticular transpira-tion. Then we should say that cuticular transpiration of these plants is in genera very high. It should be still higher at the beginning of the experiment when the lea would have a water content closer to the value at saturation.

e. Saturation deficits

As we said before, saturation deficits were determined according to Stocker's formula

$$\text{Sat. def.} = \frac{\text{Max. water content — actual water content}}{\text{maximum water content}} \times 100$$

To determine the maximum and the actual water contents we must know th fresh weight, the weight of saturated leaves and the dry weight. After weighin freshly harvested leaves (fresh weight), they were placed in a plastic bag togethe with moist filter-paper. Twenty-four hours later, new weighings gave the weigh of saturated leaves. These leaves were then sun-dried and taken to the laboratory where the drying was completed in an oven at 70°C.

We are aware that our procedure was not the most desirable, but working in th

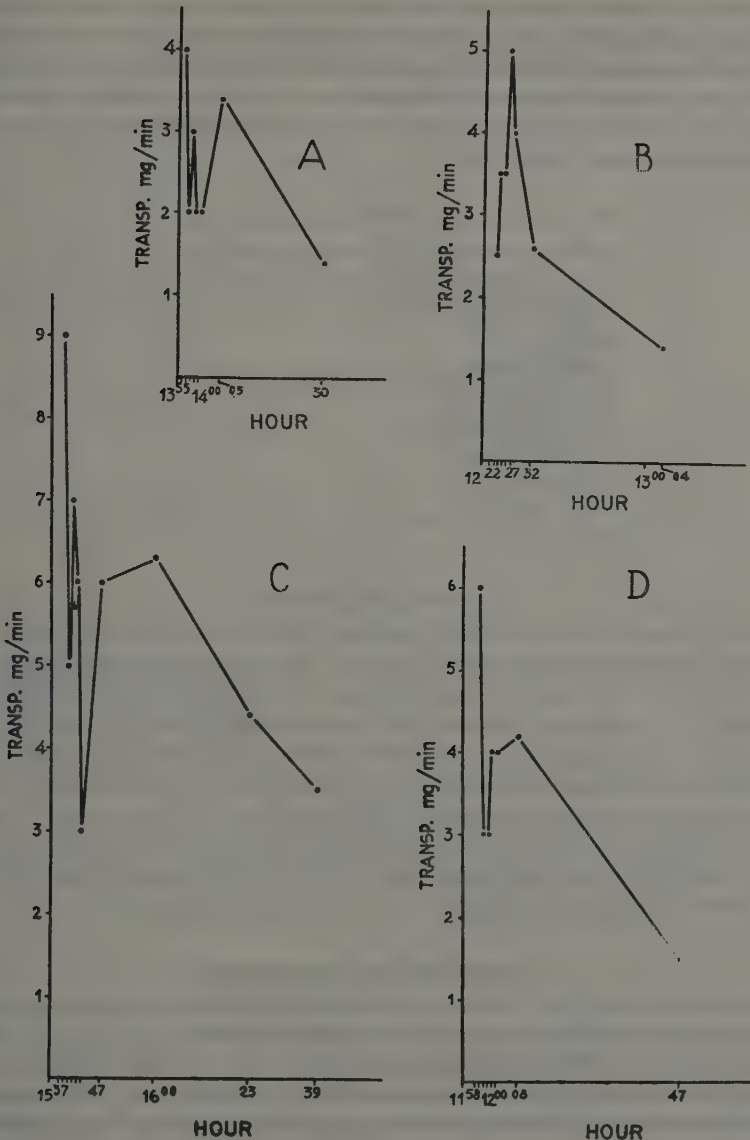

Figure 6

Curves showing stomatic reaction patterns in leaves severed from plants. A—*Discophora froesii*; B—*Sphaeradenia amazonica*; C —*Pagamea coriacea;* D—*Hevea rigidifolia.*

field, we could not do better. Our data on Table II should not be taken, then, a very accurate, but giving only an order of magnitude of the actual saturation deficits

They are in general very low, though the leaves were taken at noon-time, whe they should have the highest deficits. In only one case (*Sphaeradenia amazonica*) i is 10% of the maximum water content; in another (*Clusia spathulaefolia*) it is 6% and in all other cases 5% or less.

TABLE II

Saturation deficits in % of the maximum water content

Sphaeradenia amazonica	— 10
Maxillaria leucaimata	— 3
Zamia lecointei	— 2
Cunuria crassipes	— 1
Compsoneura debilis	— 2
Lissocarpa benthami	— 3
Retiniphyllum truncatum	— 3
Pagamea coriacea	— 5
Clusia spathulaefolia	— 6
Bactris cuspidata	— 4

f. Anatomical information

Detailed information on the anatomy of leaves of several species of the vegetatio studied, especially concerned with the stomata, will be published elsewhere. However, we wish to state here that many structures that are often connected with dr conditions, are present in this vegetation. Thus, sunken stomata, such as those o *Pagamea coriacea* (Figure 7,A and B), thick cuticle and cuticular layers, such as i *Retiniphyllum truncatum* (Figure 7,C), and *Sphaeradenia amazonica* (Figure 7,D) water-storing tissue, as in *Retiniphyllum truncatum* (Figure 7,C), mechanical tissues as in *Sphaeradenia amazonica* (Figure 7,D), etc., were found.

The meaning of such xeromorphic structures in a very humid habitat will be considered in the following chapter.

5. DISCUSSION AND CONCLUSIONS

As far as we are aware, this is the first experimental attempt to approach the problem of the ecology of the "Rio Negro Caatinga" in the Amazon.

The main problem this paper is concerned with, is why there appears such a poor vegetation in the middle of the exuberant Amazonian rain-forest, under climatic conditions which would certainly allow a much better growth. The aprioristic assumption that water could not be the limiting factor, since the yearly rainfall is enormous and since there is not a dry period during the year, cannot be made, because soils with a poor retaining capacity might not permit the storing of enough water to maintain a more exuberant vegetation, if only a few rainless days occurred.

In fact, the soils in the "caatinga" under investigation in Taraquá are very sandy, ınd thus one is entitled to expect them to have a very low water retaining power. n this way the lack of rains for a few days may bring about a pronounced dessica- ion of the superficial layers and since most of the roots are limited to such layers, he vegetation may have problems of water supply.

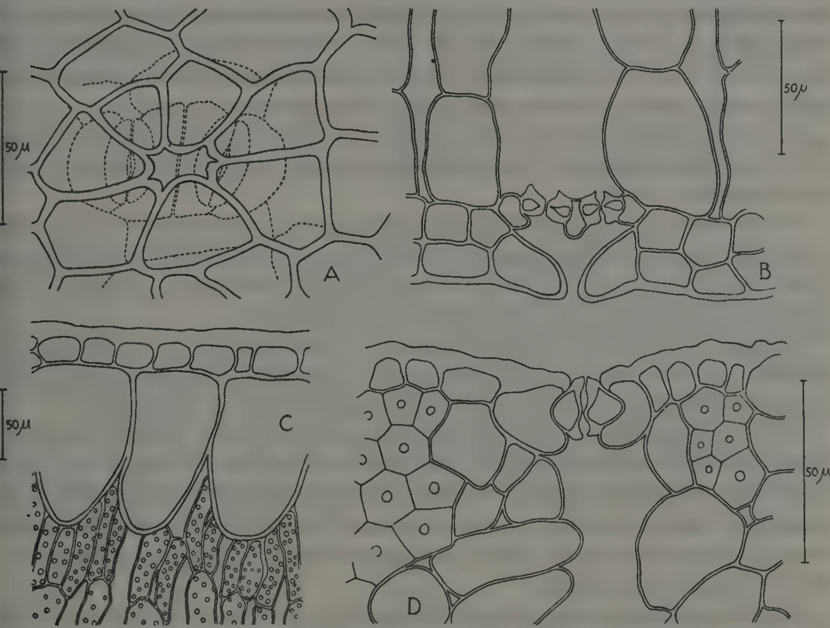

Figure 7

ome anatomical features found in the "Rio Negro caatinga". A—surface-view of the epidermis of ıe leaf of *Pagamea coriacea*. Note stomata on internal layer of the bi-serial epidermis; B—cross- ection of same leaf, passing by two stomata; C—cross-section of leaf of *Retiniphyllum truncatum*. Note thick cuticle and the large, colourless cells below epidermis; D—cross-section of leaf of *Sphaeradenia amazonica*. Note thick cuticle and sclerenchyma.

We do not wish to discuss further this problem at this moment, because we are till waiting for the results of soil analysis.

Our experimental results showed the following to be generally the case in the plants tudied: 1) They did not show a pronounced modification of the stomatic openings uring the light part of the day. 2) They did not show a restriction of transpiration hroughout the day. 3) They had high cuticular transpiration. 4) Stomatic reactions ı leaves severed from the plant were very slow. 5) The leaves of the species studied id not develop high saturation deficits.

We may now come to the conclusion, which we could not draw aprioristically: hat water is not, indeed, the limiting factor of the "caatinga" vegetation.

In fact, this vegetation, as a whole, behaved in a similar way as the "cerrado vegetation which we studied in several parts of Brazil (Ferri 1944, 1955; Ferri an Coutinho 1958) and never found to be limited by water.

In opposition, the northeastern caatingas which we have also studied (Fer 1953, 1955; Ferri and Labouriau 1952), have a quite different behaviour, as far a water relations are concerned. In fact, in these dry caatingas, the stomates close fo long periods of the day, even during the rainy season. During the dry season, the may be kept closed for days on end. Cuticular transpiration is, in general, very lo and stomatic reactions in leaves severed from the plant are very rapid.

Yet the Rio Negro Caatinga, as the cerrado vegetation in general, is rich in plan with sclerophyllous leaves. The northeastern caatinga, on the other hand, is poo in xeromorphic plants.

For this reason, even before the present investigation, we had come to understan that xeromorphy may have nothing to do with lack of water. We find in the literatu many indications that xeromorphic structures may have some connection wit nutrition problems (Grieve 1955, Killian and Lemée 1956).

In Brazil Arens (1958) attempted recently an interpretation of our data on th anatomy and behaviour of the cerrado and northeastern caatinga, on the sam ground of differences in plant nutrition between both types of vegetation.

Our present results in the Rio Negro Caatinga seem to point to a similar conclusio Since there seems to be no major water problem and since the soil is very poo xeromorphic structures which we find there should not be correlated with xer conditions, but rather with mineral deficiencies. We are soon expecting the resul of soil analysis and then we will know which are the more frequent deficiencies i the area under investigation.

Another problem we want to call attention to is the fluctuation of values in th graphs representing stomatic movements of closure in leaves taken from the plan and without water supply.

This fluctuation of values is a phenomenon which we have found many times i other plant associations, such as the northeastern caatinga (Ferri 1955, Ferri an Labouriau 1952), the cerrados (Ferri 1955, Ferri and Coutinho 1958) and also i cultivated plants, such as sugar cane (Meguro and Ferri 1956). Other authors (c Oppenheimer 1953) have observed the same fact.

We cannot believe that it is an artificial effect due only to experimental erro because it never appears in connection with some species but appears always i relation to others. We have some experiments under progress in connection wit this problem and so wish to say no more at the present time.

An intriguing behaviour was found in two Orchidaceae (*Maxillaria leucaima* and *Epistephium parviflorum*): after a distinct drop with fluctation of the water lo values, a relatively low value was reached, but it increased again, considerabl much later. This fact too is known and discussed in the literature. It was reporte by the present author in connection with the cerrado vegetation (Ferri 1955). Sinc

iere was no infiltration when the water loss increased, this effect could not be due
› a mechanical pull of the guard-cells by the neighbouring cells, which might con-
ract as a consequence of a great water deficit and determine an increase in the size
f stomatic openings.

Some biochemical transformation within the guard-cells which would bring about greater capacity of water absorption from the neighbouring cells, would also de-rmine a partial reopening of the stomata. However, on the same ground that there no infiltration at this stage, we cannot accept this idea.

Since many of these plants are sclerophyllous and have thick cuticles, the final igh values at the end of the experiments may be due to cracks opened on the urface, as a result of a great water deficit within the leaf tissues.

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REFERENCES

RENS, K., 1958, O cerrado como vegetaçao oligotrófica, *Bol. Fac. Fil. Ciênc. Letr. Univ. S. Paulo*, **224**, *Botânica*, **15**, 58–77.

ERRI, M. G., 1944, Transpiraçao de plantas permanentes dos "cerradoss", *Bol. Fac. Fil. Ciênc. Letr., Univ. S. Paulo*, **41**, *Botânica*, **4**, 159–224.

ERRI, M. G., 1953, Water balance of plants from the "caatinga". II. Further information on transpiration and stomatal behavior, *Rev. Brasil. Biologia*, **13** (3) 237–244.

ERRI, M. G., 1955, Contribuiçao ao conhecimento da ecologia do cerrado e da caatinga. Estudo comparativo da economia d'âgua de sua vegetaçao, *Bol. Fac. Fil. Ciênc. Letr., Univ. S. Paulo*, **195**, *Botânica*, **12**, 1–170.

ERRI, M. G. e COUTINHO, L. M., 1958, Contribuiçao ao conhecimento da ecologia do cerrado. Estudo comparativo da economia d'âgua de sua vegetaçao, em Emas (Est. S. Paulo), Campo Grande (Est. Mato Grosso) e Goiânia (Est. Goiás), *Bol. Fac. Fil. Ciênc. Letr., Univ. S. Paulo*, **224**, *Botânica*, **15**, 102–150.

ERRI, M. G. and LAOURIAU, L. G. 1952, Water balance of plants from the "caatinga". I. Transpiration of some of the most frequent species of the "caatinga" of Paulo Afonso (Bahia) in the rainy season, *Rev. Brasil. Biologia*, **12**, 301–312.

RIEVE, B. J., 1955, The physiology of sclerophyll plants, *Journ. Roy. Soc. Western Australia* (Presidential address 1953), **39**, 31–45.

ILLIAN, CH. et LEMEE, G., 1956, Les xérophytes: leur économie d'eau, in: *Handbuch der Pflanzenphysiologie*, Bd. III, *Pflanze und Wasser*, Springer Verlag, Berlin-Göttingen-Heidelberg, p. 787–824.

IEGURO, M. e FERRI, M. G., 1956, Economia d'âgua de cana de açûcar, *Anais Acad. Brasil. Ciências*, **28**, 523–543.

Molisch, H., 1912, Das Offen- und Geschlossensein der Spaltöffnungen, veranschaulicht durch ei neue Methode (Infiltrationsmethode), *Ztschr. Bot.*, **4**, 106–122.

Oppenheimer, H. R., 1953, An experimental study on ecological relationships and water expens of mediterranean vegetation, *Palest. Jour. Bot.*, *Rehovot ser.*, **8**, 2, 103–124.

Rachid, M., 1947, Transpiraçao e sistemas subterrâneos da vegetaçao de verao nos campos cerrad de Emas, *Bol. Fac. Fil. Ciênc. Letr.*, *Univ. S. Paulo*, **80**, *Botânica*, **5**, 1–139.

Rawitscher, F., Ferri, M. G. e Rachid, M., 1943, Profundiade dos solos e vegetaçao em camp cerrados do Brasil Meridional, *An. Acad. Brasil. Ciências*, **15**, 4, 267–296.

Stocker, O., 1929, Das Wasserdefizit von Gefässpflanzen in verschiedenen Klimazonen, *Planta*, 2–3, 278–340.

DO BIOLOGICAL TISSUES AFFECT PHOTOGRAPHIC MATERIAL? *

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ABSTRACT

Close contact between a botanical microscopic preparation and a gelatine - plate, for 30 minutes, produces on the plate an image of the preparation which shows fine details. Suitable plates are most conveniently obtained by fixation of inexposed photographic plates. The production of the chemographical image is presumably due to the enzymatic destruction of the gelatine layer of the film by the tissues of the preparation.

Herzmann et al. (1956) state that "sections of various fresh organs fixed upon photographic plates for 24 hours in absolute darkness were found to produce very distinct images of the structure of the sections. Functionally active tissues were still more photoactive. The reaction of colour plates corresponds to the natural colour of the organs used. The mechanism of this action is unknown."

I have attempted to obtain photographic images of microscopic structures invisible in an ordinary light microscope by printing them directly on very fine-grained photographic plates (Froeschel 1958). Obviously, these prints were made by exposure to light. Yet, from the above paper (Herzmann et al.) it follows that it is possible to print organic structures on photographic plates without illumination, though nothing is said as to whether the structures concerned are microscopic or macroscopic*.

I decided therefore to check the proposed method on a botanical object. The upper epidermis of the scales of *Allium cepa* was chosen for the purpose. The photographic materials used were Superchrome, Gevachrome and Lippmann plates.

Pieces of epidermis were gently pressed against the emulsion layer, or else placed in a drop of water and covered with a cover glass. In the darkroom, red or yellow light was used. The plates were subsequently placed for 6 to 24 hrs. in a wet chamber installed inside the dark room. The pieces of epidermis were then removed and the plates were developed, fixed, washed and dried.

EFFECT OF TISSUES ON SUPERCHROME PLATES

These highly sensitive plates were affected by the red light of the darkroom when the tissue was prepared and removed, and were thus blackened considerably. A first

* After conclusion of this article, I have been informed that the authors worked with 5 mm sections of animal and human organs.

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microscopic inspection of two plates which had been kept for 24 hours with a uncovered and a covered piece of epidermis, respectively, did in fact reveal a clea image of the epidermis comprising hundreds of epidermal cells, with easily visib bright lines indicating the cell walls. Careful observation shows, however, that th markings of the walls are at a level slightly different from the level of the majorit of blackened grains. Moreover, the bright cell walls are no less evident in patche where the blackened grains are scanty.

EFFECT OF TISSUES ON GEVACHROME PLATES

These plates are but little affected by red light. Macroscopically, they are onl slightly blackened. They nevertheless clearly show the image of the epidermal cell Covered and uncovered preparations were left for 24 hrs. Here, too, the image of th walls and the blackened grains are at slightly different levels, and the walls ar equally evident on patches with few and with numerous blackened grains.

EFFECT ON LIPPMANN PLATES

Lippmann plates are of very low sensitivity, and red light does not affect them. Thu neither macroscopic nor microscopic blackening occurs. Yet the image of the ce walls comes into view quite clearly, as shown in Figure 1.

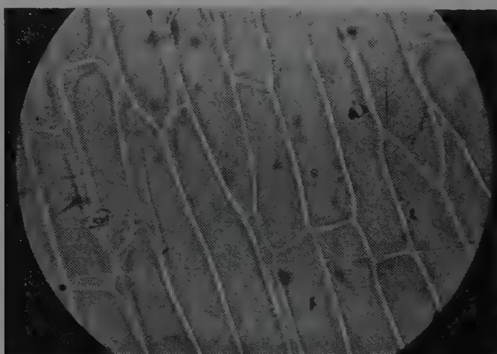

Figure 1

Photomicrograph of a Lippmann-plate, on which a naked piece of the epidermis of an onion scale wa kept for 24 hrs in complete darkness. The cell walls are reproduced; cell content is not noticeabl

These experiments raise the suspicion that the phenomenon is due solely to th presence on the photographic plate of a gelatine film and has nothing at all to d with the photosensitivity of the plate.

If this were so, it should be possible to obtain an image of the epidermis of *Alliu* even on a film of gelatine free of silver salts. Such gelatine films can be prepared b

pouring gelatine on a suitable substrate, or else by fixation of an unexposed photographic plate. Fixed plates of this kind were used to test the effect of the cell walls. With these, no dark chamber is needed, and the tests were made in daylight inside a humid chamber.

All of these experiments gave positive results, i.e. the salt-free gelatine film reproduced the epidermal cell pattern as faithfully as any unfixed photographic plate, irrespective of whether the preparation was covered or not. Nor was any difference observed between plates exposed for 24, 6 or 4 hrs. or even for 15 to 30 minutes.

To test the effect of other tissues, an onion scale was halved and the cut surface was placed on gelatine film. Within 20–25 minutes an image of the parenchymatous tissue was obtained (Figure 2).

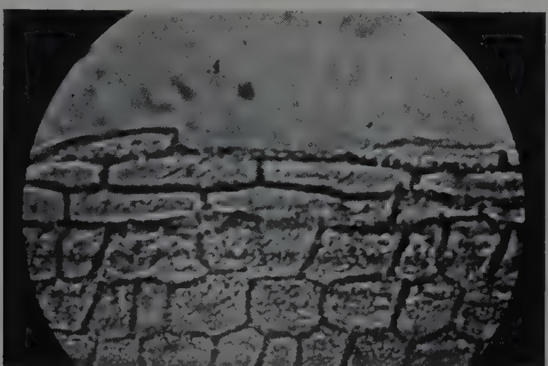

Figure 2

Photomicrograph of a silver-free gelatine film on which the freshly cut tissue of a halved onion scale had been kept in broad daylight for some 25 minutes. The images of the cell walls are evidently due to the chemical action of the walls on the gelatine. The images of the cell contents are presumably only partly due to chemical action, as the contents of some cells at least sticks to the gelatine, and has been photographed together with it. Means must still be found to rid the gelatine of adhering cell contents, so as to obtain a true picture of the chemical effect of the walls on gelatine.

Our experiments show that at least in the case of onion there does in fact exist an effect of living tissue on photographic plates, but that previous interpretations had been misleading. In any case, as far as the present experiments are concerned there can be no question of any mysterious biogenetic radiations, nor is there any photochemical process involved. Instead, there is merely the effect of biological tissue on a gelatine film, probably due to the action of enzymes. Such action is also indicated by the fact that largish pieces of onion epidermis or pieces of onion scales with freshly cut surfaces when put on a layer of gelatine 12% sink into the gelatine

within 1–2 days evidently not because of their weight, but through chemical actio on the gelatine.

Finally, it should be pointed out that gelatine films with prints of tissues keep fo months and may presumably be made into permanent preparations.

REFERENCES

1. Herzmann, J., Pospisil, J. and Stenickova, A., 1956, Influence of animal tissue on phot graphic material, *Ustav pro Peci o Matku a Dite Praha-Podoli-CSR Morf.; abstra* 1958, *Excerpta Medica*, **12.**

2. Froeschel, P., 1958, Over de shaduw van mikroskopische Preparaten, *Natuurwet. Tijdschrij*

TRANSPIRATION AND PHOTOSYNTHESIS OF DETACHED LEAVES AND SHOOTS OF *QUERCUS PUBESCENS* AND *Q. ILEX* DURING DESCCATION UNDER STANDARD CONDITIONS

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ABSTRACT

Continuous measurements were made of the transpiration and assimilation of cut-off shoots and leaves of *Quercus pubescens* and *Q. ilex* exposed to slow desiccation under standard conditions of 22°C, 10,000 lux and 550 mg/h evaporation from discs of filter-paper. Under these conditions, both total transpiration and net-assimilation of the deciduous submediterranean species *Q. pubescens* reach their highest values at a water saturation deficit of about 5.5% of saturattion weight, whereas in the evergreen Western Mediterranean species *Q. ilex* the highest values were reached at a water saturation deficit of about 8% (see Table I). The hydroactive closure of the stomata consequent upon an increase in saturation deficit reduces CO_2-absorption to a very small rate, no longer measurable. At the same time, transpiration becomes exclusively cuticular (Figure 1, 2). In *Q. ilex* closure of stomata is quicker and more effective than in *Q. pubescens*. In *Q. pubescens* stomatal transpiration and net-assimilation cease once the saturation deficit reaches 19.5–20%. In *Q. ilex*, the corresponding value is 17%. In times of drought, the xerophytic *Q. ilex* has a clear advantage over *Q. pubescens* owing to the earlier closure of stomata and the higher sublethal saturation deficit (Table II, Figure 3) which together guarantee a greater water storage. This advantage is accentuated by a very high resistance to cuticular transpiration in *Q. ilex*.

Under the conditions of our experiments the water saturation deficit at which both species of oaks attained, *with maximum water economy*, the *relatively highest* rates of assimilation ranged about 4–5% above the saturation deficit at which net assimilation rate attains the highest *absolute* values. This supports Maximov's assumption that "xerophytes may be in a position to limit transpiration in a manner that ensures the least possible interference with assimilation" (Maximov 1923, p. 137).

INTRODUCTION

he imperfect balance between water gain and water loss in a plant necessarily leads characteristic responses. Thus, as Kausch and Ehrig (1959) have recently shown perimentally, a continuously positive water balance increases shoot growth, whereas markedly negative balance, as has long been known (Maximov 1929), is reflected the reduction of the transpiring surface and in accelerated growth of roots (see so Kausch 1955). Yet even before these adaptations become effective the plant, as

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a sort of first-aid reaction, throttles transpiration by narrowing the stomata. B thus reducing transpiration, the *debit* side of the water balance is brought to a min mum. By the restoration of a more favourable balance the plant gains the time r quired to improve its adaptation to drought.

Pisek and Winkler (1953), following up Stålfelt's classical observations (Stålfe 1932, 1935) have shown that the water saturation deficit at inception of the closu of stomata and furthermore the rapidity and effectiveness of stomatal regulatio are different for different species, and are further dependent on the ecological natu of the individual plant and on the character and age of the organ concerned. Herl of shady habitats, evergreen ericaceous dwarf-shrubs, and trees, respond to an eve slightly unfavourable water balance by closing their stomata. On the other han herbs of sunny and dry habitats risk water deficits up to two times as large in ord to maintain assimilation undiminished.

As shown by Stålfelt (1935) for oats, and by Pisek and Winkler (1956) for selecte plants of different ecological types, absorption of CO_2, like stomatal transpiratio depends on stomatal aperture. Therefore, assimilation of plants subjected to a insufficient supply of water is governed by the persistent alternation between ligh controlled photoactive opening and drought-controlled hydroactive closure stomata. In addition, there is also a direct depressing effect of hydrature decrea on assimilation efficiency (cfr. Walter 1947, p. 306). Stocker, in the *Handbuch d Pflanzenphysiologie* (1956, Vol. III, p. 705–716, 0. 732) exhaustively reviews t effects of drought on assimilation and respiration. Either directly—through d hydration of the plasma—or indirectly, through closure of the stomata—a steadi rising water saturation deficit finally brings any further assimilation to a stop. Ass milation ceases before, and at most simultaneously with the cessation of stomat transpiration. Whereas water continues to escape through the cuticle, to my know ledge no uptake of CO_2 has been reported in plants with completely closed stomat leaves with closed stomata at most reassimilate their own respiratory CO_2. It is t purpose of the present study to add to the list of plants thoroughly studied by Pis and Winkler (1953, 1956) under conditions of increasing water deficit two mo species of ecological interest, the submediterranean deciduous *Queecus pubescens* a the evergreen *Q. ilex*, a representative of the Mediterranean flora with coriaceo leaves.

MATERIALS AND METHODS

The oaks used were 3–4 m high shrubs, about 20–30 years old. They grow on the we tern slope of Mt. Brione, a 376 m high hill of grey Miocene sandstones near Riva c Lake Garda. They are a few metres apart, on a 20° slope, 240 m above sea leve Owing to the mild winter climate and the brief period of summer drought prevailin in the Northern part of the Garda basin the submediterranean bush-forest of *Q pubescens—Ostrya carpinifolia* and representatives of the Mediterranean *Q. il* formation locally interpenetrate, with *Q. pubescens* confined to deep soils and

ilex flourishing on shallow soil and along rocky ridges (for a more detailed discussion of the Garda basin see Larcher, 1954).

In 1959 in the middle of July (*Q. pubescens*) and the middle of August (*Q. ilex*), 1–1.5 m long branches were cut in the early morning from a sunny part of the crown of the tree. The branches were packed in wet cloth and nylon bags, and quickly transferred to the laboratory at Innsbruck.

As both species of oak show a considerable variability in shape and character of leaves, particular care was taken to ensure the uniformity of samples used in the experiments. For *Q. pubescens* only rather small, grey-green sun leaves with acute lobes and shallow sinuses were used. The mean leaf-size (single-side) was 11.5 cm^2, and the mean water capacity, considered as water content in per cent of dry weight, was 148%. Degree of sclerophylly (Hartlaub-charakter), defined by Stocker (1931) as the ratio of dry-weight area (single-side) averaged 0.70 g/dm^2.

In *Q. ilex* all leaves of the new growth are fully developed by August, and are thus difficult to distinguish from the leaves of the previous year. Leaves of the fresh growth and leaves of the previous years were studied separately but as no important differences were found their values have been combined in the preparation of the curves. Only ovate-lanceolate, entire, dark-green sun-leaves from fruiting branches were used. Mean leaf area was 6.3 cm^2, mean water capacity 105% and mean "sclerophylly" 1.7gr/dm^2 .The branches were cut from a specimen of *Q. ilex* on rather deep soil, and for that reason presumably enjoyed a better water supply than most bushes of *Q. ilex* in the area.

In the laboratory the branches were divided, and the short shoots obtained were allowed to become saturated for 24 hours by the usual method (Stocker 1929, Pisek and Winkler 1953). Measurements were confined to terminal shoots comprising the youngest 6 nodes or their leaves.

Transpiration was measured on 4 sets of 6 leaves each, exposed at $22 \pm 2°C$ and illuminated by a xenon high-pressure lamp at an intensity of exactly 10,000 lux (= 1,000 foot candles). The lamp was an XBF 6000 of Osram (for details see Pisek and Winkler 1959). Weighing with a torsion-balance — at first at intervals of 5 min, later-on at prolonged intervals was continued until the first signs of desiccation damage became visible. Evaporation was determined by weighing discs of green filter paper 5 cm across. Throughout the period of observations, evaporation remained at 550 ± 10 mg per hour, corresponding to an evaporative power of 0.24 cm^2/h as measured with a Piche evaporimeter. Details of the technique employed have been published by Pisek and Winkler (1953).

Following Pisek and Winkler (1956), the net assimilation of 28 and the respiration of 7 shoot tips were determined by means of the ultra-red absorption recorder of Hartmann and Braun, with the xenon high-pressure lamp substituted for fluorescent tubes. All observations were made under the following standard conditions: illumination of the leaves in the container 10,000 lux; temperature of leaves $22 \pm 1°C$; air

flow: 30 l/hr i.e. 30 cm linear movement per minute; CO_2 content of air 0.033–0.035% of volume. Alternating with the assimilation tests samples were weighed at half-hourly intervals in order to determine their transpiration inside the container, in moving air. All leaves were copied on paper for planimetring. Stomata are confined to the lower side of the leaves, and leaf areas (all in dm^2) refer to one side.

RESULTS

The transpiration, in mg/g fresh weight per hour, and net assimilation, in mg CO_2 per dm^2 per hour, of *Q. pubescens* and *Q. ilex* are shown in Figure 1 and 2, respectively. Values are plotted against the corresponding values of water saturation deficit.*

As in the studies of Pisek and Winkler (1953, 1956) the curves showing the relation of transpiration and assimilation to saturation deficit agree.

Unfortunately, it was impossible to measure stomatal aperture of *Q. pubescens* and *Q. ilex* by microscopic observation or by infiltration without interfering with the experiments. However, the curves obtained permit some relevant conclusions to be drawn, as light, temperature and especially evaporation, remained constant throughout the experiments. The conversion of observed transpiration values into relative transpiration (transpiration: evaporation) was for this very reason superfluous; nor was it necessary to include data on gross assimilation. According to Pisek and Winkler (1956), as well as by our own results, dark respiration under constant temperature conditions remains constant throughout the range of deficits in which the pore closure phase in light occurs.

As may have been expected, the two morphologically and ecologically different species of *Quercus* showed characteristic differences in the inception and completion as well as in the rapidity and effectiveness of the hydroactive regulation of their stomata.

1) *Quercus pubescens* (Figure 1)

Q. pubescens is highly sensitive to water deficiency and in this respect it resembles another deciduous oak, *Q. robur*, studied by Pisek and Winkler (1953, 1956). Under the conditions of the experiment transpiration and assimilation are reduced within 20 minutes, at a water saturation deficit averageing 5.5–5.7% (Table I). After a further period of 90–100 minutes absorption of CO_2 ceases entirely, and transpiration approaches minimum values. By then, water saturation deficit reaches 19–20%.

For *Q. pubescens* the trend of the assimilation curve clearly indicates the stage at which the stomata are closed. The corresponding curve for transpiration does not provide a similar indication, as it does not show the well-known sharp bend asso-

* To facilitate comparison with the results of Pisek and Winkler, water saturation deficit is throughout calculated in per cent of saturation weight. In addition it is sometimes also shown in per cent of water capacity as in Stocker (1929).

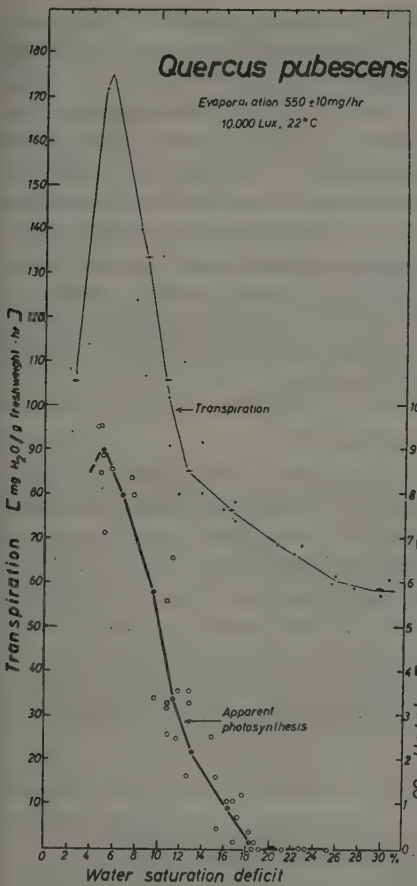

Figure 1

Course of transpiration and net assimilation of isolated leaves and shoots of *Q. pubescens* during desiccation under standard conditions. Transpiration in mg H_2O/g fresh weight per hour (dots, fine line). Assimilation in mg CO_2/dm^2 single-side leaf area per hour (circles, heavy line). Abscissa — water saturation deficit in % of saturation weight. The curves are averages based on 5–10 separate measurements (details in text).

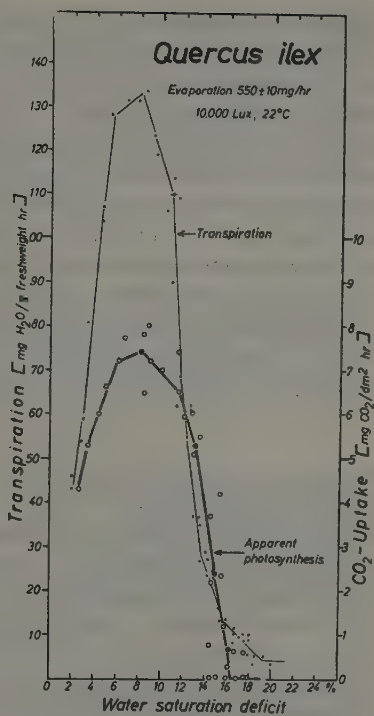

Figure 2

Relation of transpiration and assimilation to saturation deficit in mature leaves of the new growth and in one-year-old leaves of *Q. ilex*. Presentation as in Figure 1.

ciated with complete closure of stomata. This may be due to the non-simultaneous and incomplete closing of the stomata, as observed in late summer by Pisek and Berger (1938) and Pisek and Winkler (1953) in most of the broad-leaved species investigated

by them. On the other hand, cuticular transpiration is not constant. The progressive dehydration of the cuticle and of the cutin-impregnated layers of the outer walls of the epidermis reduces cuticular transpiration. According to Pisek and Berger (1938), this reduction may reach one half to one third (*Q. robur*, evergreen Ericaceae) of the initial value. Possibly incipient drying of mesophyll cell walls may be effective in the same sense (Klemm 1956). Oppenheimer (1932, 1947, 1953) has repeatedly observed incipient drying of Mediterranean evergreens to such a degree that even continuous exposure, for many hours, in an environment of high evaporative power did not lead to any further water loss. In *Q. pubescens*, once it exceeds the water saturation deficit at which effective closure of stomata is inferred from the cessation of CO_2 absorption, the cuticular transpiration — initially linked with substomatal transpiration — steadily diminishes until, at a water saturation deficit of 30% (Table II), there appear the first symptoms of desiccation damage.

2) *Quercus ilex* (Figure 2)

The coriaceous leaves of *Q. ilex* do not limit transpiration and assimilation before reaching larger deficits, of 8.0–8.2%, under experimental conditions, attained after an average exposure of 40 minutes. On the other hand, closure of stomata is more rapid. It is completed within 75–80 minutes, and the effect of closure on transpiration is much more pronounced thanin *Q. pubescens*. At a water saturation deficit of only 18.5%, transpiration of mature leaves of the new growth as well as of leaves from the previous year did not exceed 3% of the maximum value of total transpiration with open stomata. Evidently *Q. ilex*, by closing its stomata, is able to limit transpiration ten times as effectively as *Q. pubescens*.

TABLE I

Water saturation deficit, in % of saturation weight, at stomatal closure, for Q. pubescens *and* Q. ilex. (10,000 *lux*, 22°*C*, *evaporation* 550 *mg/h or* 0.24 *cm³/h* (*Brackets—extreme values*).

Plant	Stomatal closure: Deficit at inception	Stomatal closure: Deficit at completion	Highest assimilation resp. transpiration before beginning of closure	Assim. resp. transp .after completion of closure	Effectiveness of stomatal regulation as ratio: *maximum total transpiration/initial cuticular transpiration*
Quercus pubescens					
Assim.	5.5	19.5	9.05	0	—
	(5.0–7.8)	(17.5–21.5)	mg/dm² . h	—	—
Transp.	5.7	about 20	175 mg/g . h	60 mg/g . h	2.9 : 1
Quercus ilex					
Assim.	8.2	16.3	7.4 mg/dm² . h	0	—
	(6.1–8.8)	(14.5–17.8)			
Transp.	8.0	18.5	132 mg/g . h	4 mg/g . h	33 : 1
	(5.4–9.0)	(18.0–19.0)			

Kamp (1930), who has computed the ratio Total Transpiration / Initial Cuticular 'ranspiration for a series of malacophyllous and sclerophyllous plants, found values f 3.7 : 1 for *Q. sessiliflora* var. *Darwini*, for 7-month-old leaves of *Q. ilex* only .3 : 1. This should presumably be ascribed to the fact that Kamp's specimen f *Q. ilex* was grown in a green-house, which never provides the conditions conducive o the full development of xeromorphic character, since Greb (1957) was able to how that xeromorphy is primarily due to humidity stress.

DISCUSSION

n order to arrive at convenient parameters for the study of the relation between aturation deficit, transpiration and desiccation resistance of the two species of *uercus* and to facilitate comparison with previously available observations, Figure 3 vas prepared to agree with Figure 10 of Pisek and Winkler (1953, p. 273) which it fits ery well.

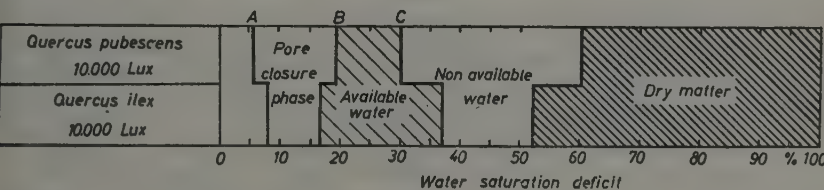

Figure 3

Vater content and dry weight of leaves, and saturation deficit at the inception (A) and completion B) of hydroactive closure. (C) sublethal deficit. All values in % weight of saturated leaf. "Available vater" refers to the amount of water storage in the leaf, which—once the stomata are tightly closed—nay be lost by cuticular transpiration without damage to the leaf. "Non-available water" refers to he residual amount of water which may only be expended under risk of severe drought damage.

Like *Q. robur*, in which—following Pisek and Winkler—stomata begin to close t a water saturation deficit of 5%, *Q. pubescens* early shows a tendency to reduce ranspiration. Yet closure proceeds over a wider range of water deficit than in *Q. obur*, in which—under comparable conditions—stomata are already completely losed by the time the saturation deficit reaches 14–15%. By its readiness to risk nuch higher saturation deficits, *Q. pubescens* is marked as well adapted to its sunny nd dry habitat (cfr. Huber 1931). Such high values of water saturation deficit at the ime stomatal closure is complete are otherwise only known for herbaceous plants of lry habitats. Such behaviour is not without its dangers, especially because the leaves of *). pubescens* are not more resistant to desiccation than most Central-European broad-eaved species. As in *Q. robur*, *Betula pendula*, *Fraxinus excelsior*, *Populus tremula* and thers (reviewed by Pisek 1956, Table II, p. 838), its sublethal water deficit ranges about 0% of the water content at saturation. Similarly, the cuticular transpiration of *Q. ubescens* is comparable to that of most Central-European broad-leaved species.

Q. pubescens is indeed unable to tolerate the continuous summer drought chara teristic of the Mediterranean climate, and even the moderate drought conditio occurring at times at the border between the *Q. pubescens* belt and the *Q. rob* zone involve a severe stress.

After four rather dry weeks of September 1958, with a total precipitation of 34 mn and maximum temperatures about 24°C, the mean water saturation deficit in leaves *Q. pubescens* had reached 68% of the value beyond which drought damage mu be expected. This is shown in Table II by values of "Trockenheitsbeanspruchung (drought-stress) defined by Rouschal (1938), Höfler, Migsch and Rottenburg (1941 as 100× the ratio between the actual value of water saturation deficit and tl saturation deficit at the time when damage by desiccation first becomes eviden Arvidsson (1951), working in Southern Sweden, finds broad-leaved trees there und a stress of only 38%.

TABLE II

Plant	Mean water-capacity (water content at saturation, in % of dry weight)	Mean sublethal deficit: in % of saturation weight	Mean sublethal deficit: in % of water content at saturation	Water saturation deficit at end of Sept. 1958: in % of of sat. weight	Water saturation deficit at end of Sept. 1958: in % of water content at saturation	Drought stress (ratio: *actual deficit/ sublethal deficit*)
Quercus pubescens	148	about 30	about 50	20.5	34	68
Q. robur (Pisek and Winkler, 1953)	—	about 28	—	—	—	—
(Arvidsson 1951)	—	—	about 50	—	—	not over 38
Quercus ilex (original data)	105	about 37	about 71	17	33.5	47
(Rouschal 1938)	—	—	62—72	—	—	mean: 43 max.: 73

The specimen of *Q. ilex* growing in the vicinity of our *Q. pubescens* was muc less subjected to drought. With a stress ("Trockenheitsbeanspruchung", see abov of only 47% it readily compares with *Q. ilex*, for which Rouschal, in Istria, foun a mean stress of 43% in July and August. The improved stabilization of the wat

balance in *Q. ilex* is due to the prompt and rapid progress of stomatal regulation once closing has begun, to the low values of cuticular transpiration (initially but one fifteenth of the "cuticular" transpiration of *Q. pubescens*) as well as to the high degree of resistance to desiccation.

The sublethal saturation deficit of 37% of saturation weight, or 71% of water content at saturation, marks *Q. ilex* as one of the highly resistant shrubs of the maquis (see Oppenheimer 1932, Rouschal 1938). Rather early complete closing of stomata in cunjunction with a higher sublethal water deficit gives *Q. ilex* a greater available supply of water than *Q. pubescens*, to be drawn upon once the stomata are closed. This emergency supply, perhaps together with its high resistance to cuticular transpiration, enables *Q. ilex* to reach "Ausdauer" (injury delay*) values 15 times as large as those for *Q. pubescens*. With evaporative power at 24 cm^3/h, the injury delay of *Q. pubescens* is only 2.7 hrs, as compared with 41 hrs for *Q. ilex*. In all of these properties, *Q. ilex* closely resembles the conifers and evergreen Ericaceae studied by Pisek and Winkler.

As stated above, in *Q. ilex* stomata become completely closed earlier than in *Q. pubescens*, suggesting that — disregarding the absolutely higher efficiency of its photosynthetic apparatus — its assimilation will exceed that of *Q. ilex* in *moderately* dry summers. On the other hand, the evergreen *Q. ilex* assimilates throughout the winter months even at the Northern limit of its distribution (Larcher 1959).

Stocker (1956, p. 732) has pointed out that the main problem concerning the competitive power of a plant in continuously dry habitats has to do with its ability "to muddle through between thirst and hunger". The ratio: Dry matter production/ Transpiration is frequently considered a measure of success in this respect. Iwanow (1913), quoted by Maximov (1923), has referred to this value as "the productivity of transpiration", calculated as gain in dry weight of the plant per kg water lost through transpiration.

The present study of the effects of various water saturation deficits does not permit the "productivity of transpiration" to be calculated. However, it does show whether total transpiration and assimilation do indeed bear the same relation to the water saturation deficit, respectively to stomatal aperture.

With evaporation, temperature and illumination at a steady value, rates of transpiration and assimilation evidently reach their peak within the same range of deficits, i.e. when stomata reach maximum apertures. Similarly, both stomatal transpiration and CO_2 absorption come to an end once the stomata are tightly closed. With the stomata more or less closed transpiration and assimilation depend mainly on the width of the stomatal apertures. Yet as long as the stomata are wide open, external conditions—as limiting factors—play the decisive role in the determination

* "Ausdauer", translated as "injury delay", is here defined as in Pisek and Berger (1938) and Pisek and Winkler (1953) as the ratio—Available water content at time of completed stomatal closure: Cuticular transpiration. It is to be considered as a kind of drought avoidance (Levitt 1958).

of the highest rates of transpiration and assimilation. This phenomenon is in accordance with Mitscherlich's law; it has been surmised by Maximov as early as 1923, and Stålfelt (1956, p. 390, 391) has discussed it exhaustively. However, transpiration and assimilation differ in their response to environmental factors. Whereas increased evaporative power accelerates transpiration, assimilation is increased by higher concentration of CO_2 in the atmosphere and by stronger illumination. As these factors are likely to vary independently of each other, it is but to be expected that the ratio between transpiration and assimilation which is most favourable fo both the water and the energy balance of the plant requires wide stomatal apertures, but not necessarily maximum apertures. Therefore, the *water economy of assimilation* may well reach its peak in the initial stage of stomatal closure, rather than at the time when the stomata are most widely open, even though the yield of the process of assimilation proper does reach its absolute peak with maximum apertures. The data presented in Figure 4 confirm this assumption.

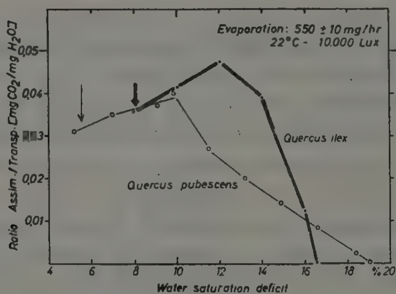

Figure 4

The relation of the "water economy of assimilation" (mg CO_2/dm^2 . h : mg H_2O / dm^2 . h) to the water saturation deficit, under standard conditions. The ratio A/T increases whenever water loss diminishes more rapidly than absorption of CO_2; it decreases when assimilation diminishes more rapidly than transpiration. *Q. pubescens*—circles, thin line. *Q. ilex*—dots, heavy line. The thin arrow indicates the beginning of stomatal closure in *Q. pubescens;* the heavy arrow—in *Q. ilex.* At the deficit values marked by the arrows transpiration and assimilation reached their absolutely highest values.

The ratio Assimilation/Transpiration (A/T = assimilation in mg CO_2 : mg water transpired) was chosen rather than "productivity of transpiration". Increase in A/T indicates increased productivity at comparable rates of transpiration, i.e. increased water economy. Whenever assimilation diminishes more rapidly than water use, this is reflected in a decrease in A/T.

All values of A/T were computed by dividing the rate of assimilation, in mg CO_2 per dm^2 of one side of leaf per hour, by the rate of transpiration in mg H_2O per dm^2 of one side of leaf per hour, determined on the same sample and at the same water

aturation deficit. Thus all measurements refer to samples in gently ventilated con-ainers and are strictly comparable. The values drawn in the figure are means of everal series of observations.

Both *Q. pubescens* and *Q. ilex* most effectively "muddled through" between thirst nd hunger when the water saturation deficit under the conditions of the experi-nent was 4–5% above the value (indicated by arrows) at which hydroactive closure f stomata begins.

Naturally, with such high deficits neither species is able to equal its assimilate-gain nder conditions of a more favorable water supply. Their rates of assimilation are ut 65% (*Q. pubescens*) and 85% (*Q. ilex*) of the highest rates possible in our ex-eriments. Yet at the same time they transpire only 48% (*Q. pubescens*) and 65% *Q. ilex*) of the amount transpired through stomata with maximum apertures. ividently, these conclusions refer to an environment at 22°C, 550 mg/h evaporative ower, 0.033–0.035% CO_2 (p.v.) and 10,000 lux. Higher evaporative power with therwise unchanged conditions would presumably shift the optimum value of /T towards higher water saturation deficits, whereas increased CO_2 and stronger llumination—without change of evaporative power—would involve a shift in the pposite sense.

At higher saturation deficits the compromise Assimilation : Transpiration again ecomes less favourable for assimilation. With stomata closing steadily, CO_2 uptake abruptly throttled, whereas owing to the persistance of cuticular transpiration vater loss is never brought to zero.

The fact that the optimum value of A/T may occur at saturation deficits which xceed the deficits which are optimal as regards the physiology of stomata has an nportant bearing on the competitive power of plants which frequently show an nfavourable water balance, or are annually exposed to a considerable water deficit or months on end, like the shrubs and trees of the Eastern Mediterranean discussed y Oppenheimer (1932, 1951). By using water economically, they are able to attain ufficient, even though not maximal, productivity. Obviously, the ratio A/T is of a nore economical than ecological significance for the numerous plants which may ever suffer a shortage of water throughout their period of activity. The daily trend f the ratio A/T of the latter plants is mainly influenced by the different response of ssimilation and transpiration to environment factors than by hydroactive stomatal egulations (Koch 1957).

ACKNOWLEDGEMNTS

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REFERENCES

Arvidsson, I., 1951, *Oikos, Acta oecol. scand. Suppl.*, **1**, 1.
Greb, H., 1957, *Planta*, **48**, 523.
Hoefler, K. , Migsch, H. and Rottenburg, W., 1941, *Forschungsdienst*, **12**, 50.
Huber, B., 1931, *J. Ecol.*, **19**, 283.
Kamp, H., 1930, *Jb. wiss. Bot.*, **72**, 403.
Klemm, G., 1956, *Planta*, **47**, 547.
Koch. W., 1957, *Planta*, **48**, 418.
Kausch, W., 1955, *Planta*, **45**, 217.
Kausch, W, and H. and Ehrig, A., 1959, *Planta*, **53**, 434.
Lange, O. L., 1957, *Ber. d. D. B. G.*, **70**, 31.
Larcher, W., 1954, *Planta*, **44**, 607.
Larcher, W., 1959, *Ber. d. D. B. G.*, **72**.
Levitt, J., 1958, Frost Drought and Heat Resistance, *Protoplasmotologia*, VIII, 6. Springer, Wie
Maximov, N. A., 1923, *Jb. wiss. Bot.*, **62**, 128.
Maximov, N. A., 1929, *The Plant in Relation to Water*, Allen & Uwion, London.
Oppenheimer, H. R., 1932, *Ber. d. D. B. G.*, **50 a**, 185.
Oppenheimer, H. R., 1947, *Pal. J. Bot. ,Rehovot Ser.*, **6**, 63.
Oppenheimer, H. R., 1951, *J. Ecol.*, **39**, 356.
Oppenheimer, H. R., 1953, *Pal. J. Bot., Rehovot Ser.*, **8**, 103.
Pisek, A., 1956, in: *Ruhland's Hdb. d. Pfl. Phys.*, **3**, 825–853, Springer, Berlin, Goettingen, Heide berg.
Pisek, A. and Berger, E., 1938, *Planta*, **28**, 124.
Pisek, A. and Winkler, E., 1953, *Planta*, **42**, 253.
Pisek, A. and Winkler, E., 1956, *Protoplasma*, **46**, 597.
Pisek, A. and Winkler, E., 1959, *Planta*, **53**, 532.
Rouschal, E., 1938, *Jb. wiss. Bot.*, **87**, 436.
Stalfelt, M. G., 1932, *Planta*, **17**, 22.
Stalfelt, M. G., 1935, *Angew. Bot.*, **17**, 157.
Stalfelt, M. G., 1956, in: *Ruhland's Hdb. d. Pfl.-Phys.*, **3**, 342–350, 351–426, Springer, Berli Goettingen, Heidelberg.
Stocker, O., 1929, *Planta*, **7**, 382.
Stocker, O., 1931, *Jb. wiss. Bot.*, **75**, 496.
Stocker, O., 1956, in: *Ruhland's Hdb. d. Pfl.-Phys.*, **3**, 696–741, Springer, Berlin, Goettinge Heidelberg.
Walter, H. 1947, Einführung in die Phytologie I. Ulmer, Stuttgart.

CHILLING AND FROST DAMAGE IN BANANA LEAVES*

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ABSTRACT

The banana is a frost tender plant. Frost injury in the leaves begins at a temperature of $-1.5°C$ to $-2.0°C$, a duration of 10–15 minutes being sufficient to cause immediate frost damage. Osmotic pressure in leaves not injured by frost, never exceeded 15 atm. Upon the occurrence of frost injury, it rose to 17–21 atm. just prior to the death of the leaves. Chilling damage occurred at temperatures close to 0°C. The injury which manifests itself by yellowing and watery spots, appears gradually and seems to be connected with the age of leaf and plant. The appearance of these symptoms is followed by an increase in the osmotic pressure of affected parts. In fruit-bearing plants, chilling leads to accelerated senescence and early death of the leaves. The mature leaves of suckers suffer localized injury but may recover. The relation between the high sensitivity of the banana plant to drought and to low temperatures is discussed and it is suggested that a similarity exists between chilling injury and senescence.

The problem of the resistance of plants to extreme temperatures and drought has een reviewed by Levitt (1956) in a monograph. The work of Asahina (1956) has dvanced the understanding of the mechanism of frost damage. The damage is nown to be due to the formation of ice crystals in the plant tissues. Ice formation egins in the intercellular spaces and may also advance into the cells themselves. The damage is caused mainly by desiccation of the cells as water is withdrawn while ce is being formed.

The nature of damage by chilling is insufficiently understood. Lieberman et al. 1958) have shown that chilling is accompanied by a large increase in the permeability f the plasmatic membranes.

The banana plant is known to suffer severely from frost. For example, in Israel, n 1956/57, frost damage resulted in losses of IL 1.5 million, and again, in 1958/59 osses from frost damage amounted to IL 2.5 million. The 1958/59 season was one f the severest frost seasons on record in Israel. Frosts began early, around the end f November, and there were altogether thirteen frost nights in the Southern Coastal Plain. On nine nights the temperature dropped below $-1.5°C$. During the 1957/58 nd 1958/59 seasons sprinkler irrigation and artificial fog were investigated as possible nethods of reducing frost damage (Shmueli et al., 1959).

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The present paper is concerned with the effect of temperatures below 0°C o banana leaves.

MATERIALS AND METHODS

Data were obtained from the following banana groves:

A — Kvutzat Schiller, where protection by sprinkling was carried out in 1957–9
B — Carmia, where a similar experiment was performed in 1958/59.

Groves A and B were situated in the southern part of the Coastal Plain, wher temperatures several degrees below zero are known to occur, on the average, onc in two or three years.

C — A number of unprotected groves in the northern part of the Coastal Plai and in the Jordan Valley, where temperatures below 0°C occur only about onc every five years.

In grove A, plant temperatures were measured by means of 42 specially devised fixe thermocouples, which were set up by the Research and Extension Department of th Israel Meteorological Service (Gilead and Rosenan, 1957). The locations of the thermo couple points in relation to a banana stoolbed (one fruit-bearing plant and on

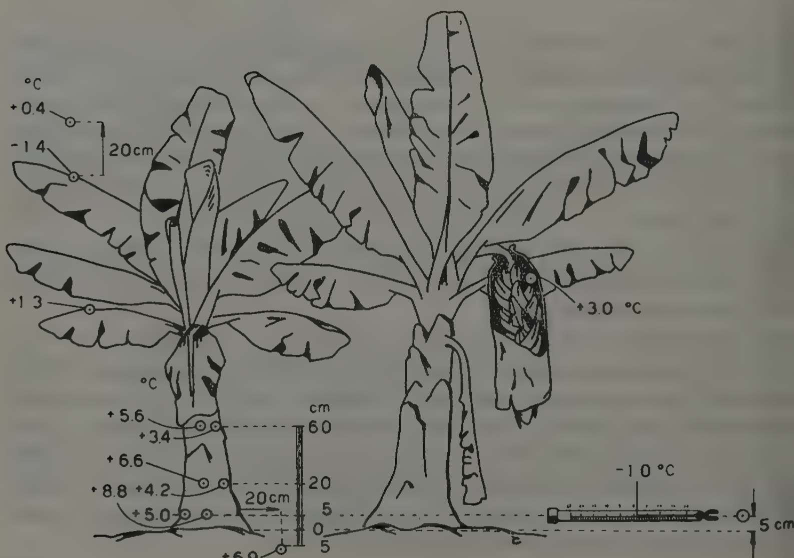

Figure 1
Kvutzat Schiller, 4.2.58. 06.00 hr. Diagram illustrating the temperatures in a banana stoolbed on light frost night. (At the right – the "grass-minimum")

sucker) is illustrated in Figure 1. For leaf temperature determinations, the thermocouple points were inserted into the upper side of the midrib to a depth of 2 mm. On the selected upper leaf, the measurement was always made on a fully exposed, horizontal part of the midrib. The lower leaf was in each case protected by another, overhanging leaf.

Two measurements were made at each of the three levels chosen on the pseudostem (see Figure 1), one in the centre and the other 5 mm below the surface.

Leaf and pseudostem temperature measurements were made in five and three stoolbed replications, respectively. In addition, "grass-minimum" temperatures were recorded at 5 cm above open fallow ground, at a distance of about 20 m from the grove.

In groves B and C only "grass-minimum" temperature measurements were made.

Regular observations were made in groves A and B, on about 1,500 stoolbeds, which had been chosen in advance. Damage symptoms (colour changes, necrosis, death) of leaves were noted.

The changes in leaves induced by low temperatures were striking and uniform throughout the groves, and therefore typical pictures (Figures 3 – 8) rather than numerical data are presented.

Osmotic pressure of leaves was determined cryoscopically (Walter 1931). Each determination was replicated on four samples, taken between 11.00 hr and 13.00 hr. Each sample consisted of fragments of five leaves. In certain cases of doubt as to the viability of leaf tissues, plasmolysis tests were made using 0.7 M mannitol solution.

THE TEMPERATURE OF BANANA LEAVES IN RELATION TO FROST DAMAGE

A detailed report on leaf temperatures measured during frost nights will be published elsewhere (Shmueli and Manes). Selected data, directly concerned with the appearance of frost and chilling damage, are briefly reported below.

The lowest temperature of the plant occurs on the exposed upper horizontal leaf. The temperature of this leaf is very close to the "grass-minimum" temperature (Figure 1). The difference between the two readings never exceeded 0.8°C. Thus, the "grass-minimum" thermometer on open ground affords a good indication of the temperature of the topmost horizontal leaf. Differences between simultaneous temperature readings on five uppermost horizontal leaves were invariably small and the maximum range did not exceed 1.2°C. Nevertheless, these small differences were often found to determine the ultimate fate of the leaf. For example, Figure 2 shows the nocturnal temperatures of three horizontal topmost leaves, and of the air temperature 20 cm above them. The part of the curve showing the temperature changes of the "cold" leaf around 06.00 hr. is similar to the curves showing the temperatures in plant tissues during freezing and ice formation (see Figure 2 in Lewitt 1956). At 06.00 hr. we found ice crystals on the surface of the "cold" leaf, while only dew was found on the surface of the other leaves.

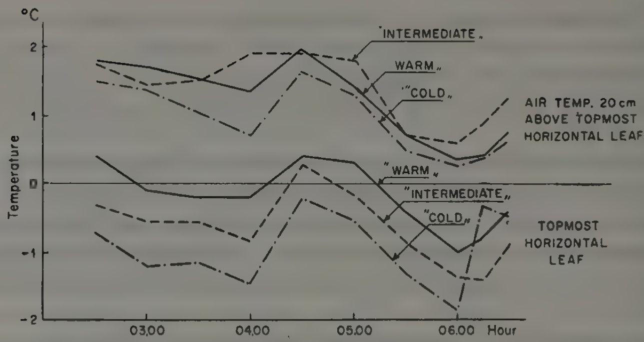

Figure 2

Kvutzat Schiller, 4.2.58. Temperature curves for air and for the topmost horizontal leaf.

Seven hours after the frost, tests for plasmolysis showed that in the "cold" leaf blade, near the point where the temperature dropped below $-1.5°C$, most cells were dead. On the other hand, in leaves where temperatures remained above $-1.5°C$ ("intermediate" $-1.4°C$ and "warm" $-0.9°C$, in Figure 2), cells showed normal plasmolysis. Two days after the frost large, parts of the "cold" leaf were already dead, while in the "intermediate" leaf no damage symptoms appeared (compare Figure 2 and Figures 3 and 4).

In all cases where temperatures below $-1.5°C$ occurred in the upper horizontal leaves, injury was visible the following morning. No immediate damage was observed in leaves whose temperature remained above $-1.5°C$.

It is possible to conclude that frost damage appears in those tissues of the topmost horizontal leaves in which the temperature drops to between $-1.5°C$ and $-2.0°C$. As can be seen in Figures 2 and 4, exposure to this critical temperature for only a short time is sufficient to bring about death of the cells. In all cases when temperature in the topmost horizontal leaves dropped below $-1.5°C$, even for a period of 10–15 minutes, necrotic areas came to be seen in the leaf-blade.

During frost nights on which a temperature of $-2.0°C$ or below is maintained for prolonged periods of time, damage is caused to the entire surface of all the exposed leaves. The leaves are almost completely killed by the frost and the grove is severely damaged (Figure 5).

However, even on nights when the temperature of exposed leaves drops to $-2.0°C$ or below, the temperature in the pseudostem and of the protected leaf parts (the leaf-blade around the base of the leaves and the covered parts of the youngest rolled leaf), remains about 0°C. Nevertheless, the cells of these leaf-blade parts are severely injured and die within a few days following the frost night. Suckers, on the other hand, do not generally die. Even after a winter with many serious frosts, as in 1956/57 and 1958/59, most of the suckers renewed their growth in the spring.

FREEZING POINT AND OSMOTIC PRESSURE OF CELL SAP

Representative data of the osmotic pressure of the banana leaves in winter are pre-ented in Tables I–III. Variability between replications was small. The mean standard rror for all the determinations presented in Tables I – III is ± 0.62 atm. The freezing oint of the cell sap from the topmost horizontal leaves of suckers was between -1.0°C and -1.2°C. It seems that frost injury begins in banana leaves at a tempera-ure about 0.5°C lower than the freezing point of expressed cell sap.

In the sucker, osmotic pressure is lowest in the youngest rolled leaf (see Table I) nd gradually increases from the first open leaf to the third or fourth leaf (the top-nost horizontal exposed leaf). The osmotic pressure decreases again in the older eaves. The osmotic pressures in the leaves of the fruit-bearing plant are similar to hose found in the horizontal leaves of the sucker.

TABLE I

Osmotic pressure (atm.) of banana leaves in winter (Kvutzat Schiller — grove A)

Date	Fruit-bearing plant, upper horizontal leaf	Sucker			
		Youngest rolled leaf	First open leaf	Upper horizontal leaf	Lower horizontal leaf
7.12.57	11.36	**	9.44	11.64	8.67
2. 2.58	12.92	**	10.40	12.84	10.28
6. 2.58	12.36	8.43	10.42	13.18	**
6. 2.58	13.96	8.83	9.96	14.16	10.92
5. 3.58	11.08*	8.91	11.44	12.64	11.08
8.12.58	12.92	8.19	12.04	13.12	9.39

* — Leaf base prior to complete drying up. ** — Not sampled.

It may also be seen from Table I that osmotic pressure varies very little during he winter and is similar to the summer level (Shmueli 1953). In banana leaves un-njured by frost, osmotic pressure never exceeded 15 atm. However, much higher osmotic pressures of 17 – 18 atm. may be reached in the green bases of leaves damaged y frost (see Table II).

Banana leaves attained even higher osmotic pressures following the occurrence of an unusual snowfall in the coastal and interior plains of Israel in 1950. On February , 1950, two days after the snow had thawed off the leaves, osmotic pressures close o 21 atm. were recorded in groves in the Jordan Valley. The green bases of the leaves, which had not displayed any immediate visual symptoms of damage died within a

few days after the frost. Sap analysis on February 8, 1950, showed that the quantity of reducing sugars was approximately double the normal amount. Absence of an increase in osmotic pressure under normal winter conditions, and the sharp rise in reducing sugars and osmotic pressure following frost—ending in death of the leaves—establishes that the banana is a frost tender plant.

CHILLING DAMAGE

The nature and extent of chilling damage could be clearly observed in the experimental groves A and B (Kvutzat Schiller and Carmia) in 1958/59, which were sprinkled during frost nights. During the frost nights at the end of November 1958, sprinkling maintained the leaf temperatures above $-1.0°C$ even when the temperature of unsprinkled leaves in these groves dropped to $-3.0°C$. Consequently, the sprinkled leaves did not die, and during the first few days following the frost no external or internal symptoms of damage appeared (Figure 6 and Table II). However, 4–5 days

TABLE II

Osmotic pressure (atm.) of banana sucker leaves from sprinkled and unsprinkled plots, on the frost nights 25–26.11.1958 (*Kvutzat Schiller — grove A*)

Date	Sprinkled Plants		Unsprinkled plants	
	Youngest rolled leaf	Upper horizontal leaf	Youngest rolled leaf	Upper horizontal leaf
26.11.58	6.14	10.60	7.02	14.56*
27.11.58	6.39	11.32	9.15	16.82*
28.11.58	7.71	10.24	9.15	17.32*
5.12.58	7.48	9.87	8.55	8.92**

* Parts of leaf-blade taken from leaves most of whose blade suffered frost damage.

** Parts of first leaf unrolled after the frost.

after the chilling, the colour of the leaves began to change from dark green to light green.

Under normal conditions, such a change of colour occurs in old leaves before death. In the present case, the rate of colour change was more rapid in the fruit-bearing plants than in the suckers, which had not yet flowered. A week after the chilling, yellow areas began to appear on the leaves. Watery spots developed within these areas owing to the filling of the intercellular spaces with liquid. Within ten to twenty

Figure 3

Kvutzat Schiller, 6.2.58. Leaf whose temperature dropped to $-1.4^{\circ}C$ on night of 4.2.58 (see Figure 2 – "intermediate").

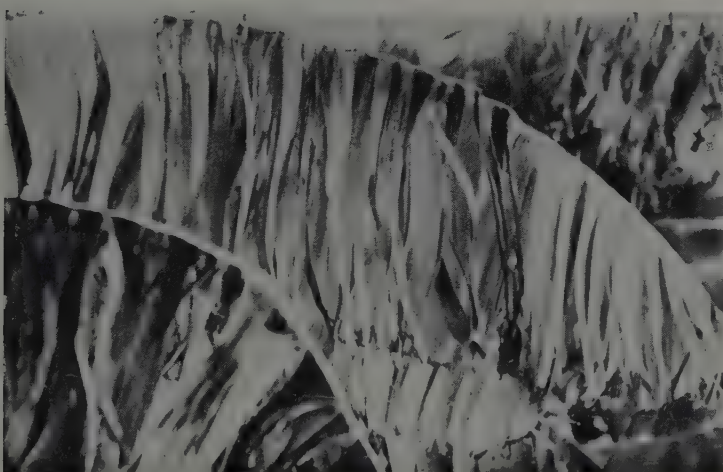

Figure 4

Kvutzat Schiller, 6.2.58. Leaf whose temperature dropped to $-1.8^{\circ}C$ on the night of 4.2.58 (see Figure 2 — "cold").

Figure 5:

Carmia Grove. 2.12.58. View from tower of plot which was not sprinkled on the frost nights of 25-26.11.58.

Figure 6:

Carmia Grove. 2.12.58. View of adjacent plot which was sprinkled during the frost nights of 25-26.11.58.

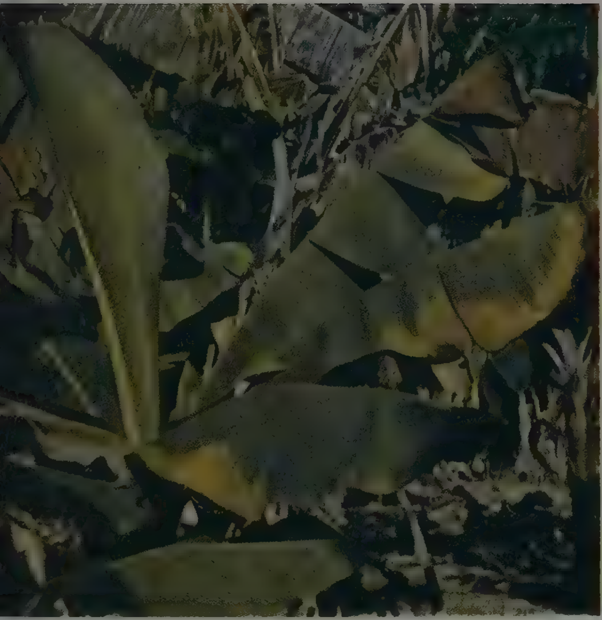

Figure 7:

Carmia Grove. 16.12.58. Typical chilling symptoms on leaves of suckers. On the second leaf from the bottom of the picture, watery spots are visible within the yellow area. Actually, there were more such spots than can be seen in the reproduction.

Figure 8:

Carmia Grove. 16.12.58. Case of extreme chilling damage appearing in leaves with blades nearly vertical during the frost nights.

days after chilling, the yellow colour had extended over the entire leaf area of the fruit-bearing plants and the leaves began to die.In these leaves, osmotic pressure reached particularly high values (Table III, yellow leaf, 16.12.58). Within a month, almost all the leaves of the plants of the older generation were dead. About two weeks before death the osmotic pressure of leaves dropped (see Table III—yellow leaf). In summing up we can say that the chilling led to accelerated senescence and early death of the leaves of the fruit-bearing plants.

A different process was observed in the leaves of the suckers. Within 2–3 weeks after chilling, areas with a green and yellow pattern (see Figures 7 and 8), and with watery spots, developed in these leaves. The yellow patches slowly extended over limited areas of the leaf blade. After the fourth week, no further extension occurred. Furthermore, the watery spots within the yellow areas gradually disappeared, and the colour of the leaves became darker and almost uniform.

About three weeks after chilling, at the time when the variation between green and yellow areas on the sucker leaves was most pronounced (see Figures 7 and 8), a marked difference in osmotic pressure was also recorded. The yellow areas had a higher osmotic pressure, of 3–4 atm., than the green ones (see Table III, 16.12.58 and 12.3.59). This difference disappeared two weeks later.[a]

TABLE III

Osmotic pressure (atm.) of banana leaves from plots sprinkled on frost nights of 25–26.11.58 *at Carmia—grove B, and at Kinneret — grove C, which suffered from chilling in Feb.* 1959

Grove and date	Fruit-bearing Plant, Yellow leaf	Sucker		
		Leaf with green and yellow areas		Green leaf
		Yellow areas	Green areas	
B*, 16.12.58	15.92	15.70**	12.96	13.60
B , 31.12.58	9.69	12.52	13.20	12.20
B , 14. 1.59	9.97	13.12	12.45	10.28
C , 12. 3.59	***	13.12	9.27	**

* The consecutive samples originate from the same leaves.
** In the transition zone between green and yellow areas osmotic pressure was 15.04 atm.
*** No such leaves occurred on sampling date.

Changes similar to those described were also observed in December, 1958, in the Coastal Plain and in March, 1959, in the Jordan Valley in a number of groves not protected by sprinkling (groves C). These groves were located in warmer areas than

[a] (Added in print, 1.3.1960. Similar results were obtained in further observations during the 1959/60 season, on three occasions in late November, two in early December and two frost nights in January. E.S.)

the sprinkled ones (groves A and B). On the basis of measurements of "grass-minimum" temperature in groves C, we may assume that the temperature of the upper horizontal leaves did not fall below $-1.5°C$. The appearance of chilling damage both in the sprinkled groves in the "cold" locations and in the unsprinkled groves in the "warm" locations indicates that there is no definite relationship between sprinkling and the appearance of chilling damage in the leaves (yellow areas). The direct cause of chilling damage is the occurrence of a certain temperature below 0°C. In the "cold" groves (A and B) the sprinkling produced temperatures which resulted in a general and uniform occurrence of chilling damage, while in the unsprinkled, "warmer" groves (C) such damage appeared only on those leaves which suffered temperatures sufficiently low to cause chilling damage. As regards to the location of the yellow areas in sucker leaves, a consistent difference was found between sprinkled and unsprinkled groves. In the sprinkled groves, the yellow areas occurred on the leaf margins (see Figures 7 and 8), whereas in unsprinkled groves they appeared on the central part of the leaf, near the midrib. These differences in the location of the damaged area seem to permit some conclusions to be drawn regarding the temperature at which chilling damage occurs. On unsprinkled leaves, the vertical, drooping margins are warmer by 0.5–1.0 degrees than the median horizontal parts. The reverse may be true for sprinkled leaves, in which the vertical margins receive less water, and are thus probably colder than the horizontal areas. It may, therefore, be conjectured that the temperature which causes chilling damage in mature leaves is approximately $-1.0°C$. This is corroborated by the fact that in the sprinkled groves, where the temperatures in horizontal leaves on the frost nights were about $-1.0°C$, all exposed mature leaves showed typical chilling damage symptoms. Our data are not sufficient in order to determine the minimum time duration of critical temperature for chilling damage, but it seems that it is longer than that which causes frost damage at a lower temperature.

DISCUSSION

Banana leaves are very sensitive to low temperatures, presumably owing to the combined effect of their morphological, anatomical, and physiological properties. The leaves are very large, and have extremely large cells and intercellular spaces (Skutch 1927). The water content of the leaves is high, and they are very sensitive to change in the degree of hydration (Shmueli 1953). It has also been found that the osmotic pressure is low, and the freezing point of the expressed cell sap is correspondingly high. All of these factors facilitate initiation of frost injury in the leaf tissues at a temperature of -1.5 to $-2.0°C$, and at the same time help to explain the rapid and fatal damage. This work shows that the banana is a typical frost tender plant. The banana, as like other frost tender plants (see Levitt, 1956), is also very drought sensitive. The osmotic pressure is a good indication of its lack of hardiness. The osmotic pressure is low throughout the year, with but small variations due to

seasonal changes in temperature and soil moisture. The maximum values of the osmotic pressure in the leaves do not as a rule exceed 15 atmospheres. Osmotic pressure values of 17–21 atm. were occasionally observed, accompanied by high concentrations of reducing sugars. This always occurred immediately following frost damage, just prior to the death of the leaves.

Following chilling, changes also appear in the osmotic pressure of the cell sap. These are also apparently related to increased hydrolytic activity, and the resultant increase in sugar concentration. These changes do not take place immediately but develop slowly and are most pronounced about twenty days after chilling. Yet, even before this, changes in the appearance of the leaf indicate decomposition of chlorophyll and an increase in the permeability of the cell membranes. The rate of these changes is clearly related to the age of both the leaf and the whole plant. Partial destruction of the chlorophyll and the consequent change of colour from dark to light green occurs already during the first week after chilling; it occurs in the leaves of both the fruit-bearing plant and the sucker. However, the youngest leaf of the suckers appears to be resistant to chilling, since it does not show colour changes.

The patchwork of green and yellow areas with watery spots appeared in all mature leaves during the second week after chilling. In the leaves of fruit-bearing plants, the yellow colour spread over the whole leaf area within twenty days, and within another week or two the leaves died. This process was much faster than the normal rate of ageing. Were it not for the chilling, such leaves would have remained alive longer by one to two months. On the leaves of suckers the patchwork of green and yellow areas was most pronounced in the third week after chilling. By then, the leaves also showed considerable differences in osmotic pressure between areas of different colouring. This abnormal condition does not spread over all the leaves of the sucker. Eventually, it recedes and the leaves may even recover.

The mechanism leading to chilling damage is not yet properly understood (Levitt 1956). An evaluation of the facts presented here may aid in clarifying the processes related to chilling damage. The injury appears gradually, and the extent of the damage is a function of the age of the leaf and of the plant. In fruit-bearing plants chilling led to accelerated senescence and early death of leaves. The mature leaves of suckers also suffer injury, but may recover. The youngest, rolled leaf does not suffer from chilling damage. Chilling injury results in decomposition of chlorophyll and increased permeability of the cell membranes. Following this, the osmotic pressure of affected parts rises.

It appears that the changes observed in the leaves of the banana following chilling suggest that chilling causes physiological changes similar to those of senescence. A number of facts which came to light during recent years tend to confirm the above hypothesis:

Weybrew (1957) observed in all the tobacco varieties tested by him that the chloroplasts of immature leaves are relatively more resistant to deterioration caused by

external conditions than those of mature leaves. Lieberman et al. (1958) have shown conclusively that chilling damage is related to an increase in the permeability of cell membranes. The hypothesis of physiological similarity between chilling damage and natural ageing is further supported by the work of Sacher (1957, 1959) which established that: 1) senescence is accompanied by an increase in permeability; 2) increase of permeability and senescence can be largely prevented by the addition of auxins. The results obtained by Crane (1954) hint at the possibility of diminishing chilling injury by the use of plant growth regulators.

The hypothesis of physiological similarity between chilling damage and senescence requires thorough experimental investigation. It is possible that such research may also provide methods of preventing damage by chilling.

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REFERENCES

Asahina, E., 1956, *Contributions from the Institute of Low Temperature Science*, **10**, Japan.

Crane, J. C., 1954, Frost resistance, and reduction in drop of injured apricot fruits effected by 2, —4, —5 trichlorophenoxy-acetic acid, *Proc. Amer. Soc. Hort. Sci.*, **64**, 225–231.

Gilead, M. and Rosenan, N., 1957, Micro-Climatological observations in a banana plantation, *Journ. Meteor. & Geophys.*, **8**, 1–5.

Levitt, J., 1956, *The Hardiness of Plants*, Academic Press, New York.

Lieberman, M., Craft, C. C., Audia, W. V. and Wilcox, M. S., 1958, Biochemical studies of chilling injury in sweet potatoes, *Plant Phys.*, **33**, 307–311.

Sacher, J. A., 1957, Relationship between auxin and membrane-integrity in tissue senescence and abscission, *Science*, **125**, 1199–1200.

Sacher, J. A., 1959, Studies on auxin–membrane permeability relations in fruit and leaf tissues, *Plant Phys.*, **34**, 365–372.

Shmueli, E., 1953, Irrigation studies in the Yordan Valley. I. Physiological activity of the banana in relation to soil moisture, *Bull. Res. Counc. of Israel*, **3**, 228–247.

SHMUELI, E., MANES, E., RAVITZ, E. AND MILLS, D., 1959, Prevention of frost damage in banana groves. First report. *Div. of Publ. Beit-Dagan, Spec. Bull.* 23.

SHMUELI, E. AND MANES, E., 1960, *Ktavim*, in press.

SKUTCH, A. F., 1927, Anatomy of leaf of banana *Musa sapientum* L. var. hort. Gros Michel, *Bot. Gaz.*, **84**, 337–391.

WALTER, H., 1931, *Die Hydratur der Pflanze*, Fischer, Jena.

WEYBREW, J. A., 1957, Pigment changes in tobacco during natural and induced senescence, *Tobacco* **144**, 6–8.

DIURNAL FLUCTUATIONS IN WATER BALANCE FACTORS OF GLADIOLUS LEAVES

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ABSTRACT

1. The daily fluctuations (during 24 hours) of the many factors determining the plant water balance were recorded in gladiolus leaves by hourly simultaneous determination of transpiration, stomatal aperture, water saturation deficit (WSD), osmotic values, water content and meteorological data.

2. The daily curve of transpiration generally followed that of meteorological conditions.

3. The rate of transpiration was found positively correlated to the stomatal aperture to a width of 4 microns.

4. A surprising opening of the stomates in the two lower leaves was observed before midnight, after the stomata had been closed for a few hours in the early part of the night

5. Data was presented indicating that the previously reported decrease in water content during the late hours of the night was caused, not by diminishing water, but by increasing dry weight.

6. The WSD of the 4th leaf was at all times significantly higher than that of the third leaf.

7. Osmotic values during the day were positively correlated to the WSD and negatively to the water content. During the night, however, they seemed to be more influenced by changes in the content of osmotically active substances.

8. The practical consequence of this investigation was that the most suitable time to test the leaf water balance factors, as a possible physiological indication of irrigation requirements, is three hours after sunrise.

INTRODUCTION

Diurnal curves of various factors determining plant – water balance have been established in many plants. Numerous transpiration curves have been recorded of different plants and under varying circumstances. Most of these works were reviewed by Burgerstein (1925), Maximov (1929), Crafts et al. (1949), and lately by Stocker (1956) and Stålfelt (1956). Several workers investigated the changes in stomatal aperture as well, while others studied this factor alone.

The diurnal fluctuations in water content were studied by Stanescu (1936), Portsmouth (1937), Kramer (1937) and Wilson et al. (1953).

Many authors investigated the water saturation deficit (WSD) of the leaf, but a few of them named or calculated this factor differently. Therefore, one should be careful when comparing data of one author to those of another. Daily march of WSD was established by Maximov (1929), Stocker (1929), Compton (1936), Runyon (1936), Weatherley (1951), Morello (1953) and Nitzan (1954).

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Daily curves of variations in osmotic values (OV) were determined by Beck (1931), Herrick (1933), Stoddart (1935), Runyon (1936), Marsh (1940) and Shmueli (1951).

Some of these workers studied not only one factor but several, often with reference to the meterological data. Probably the greatest number of simultaneous measurements of these factors was recorded by Oppenheimer and Mendel (1939). Their curves include data from daylight hours only, however, and the WSD and OV rates were determined by them only once in four hours.

Thus, so far as we know, none of the numerous works in this field includes complete diurnal curves of all the above mentioned water balance factors, determined simultaneously in any plant organ. Having established many daily curves of one or more of these factors in different parts of gladiolus plants under various conditions (Halevy 1958), we felt that some of the diurnal fluctuations of these rates could be better understood when compared to changes in other factors occurring at the same time. This was one purpose of the present investigation.

In addition, this experiment had a practical purpose—to find the most suitable hour to test the influence of soil moisture stress on the plant water balance, as a basis for studies in physiological indications for irrigation of gladiolus.

MATERIALS AND METHODS

The experiment was carried out on April 23, 1957, with the gladiolus variety "Snow Princess", grown in loamy sand soil. A day before the experiment, a heavy rain fell, leaving the soil saturated. Determination of the soil moisture had shown that the soil was very near to field capacity, with soil moisture tension at 0.35 atm. Only plants of similar developmental stage were investigated; each of them had 5 fully developed leaves.

The experiment started at 7 a.m. and continued till 8 a.m. the next day, i.e. 25 hours. The following factors were determined every hour by three observers working simultaneously.

1. *Transpiration* was determined by rapid weighing of detached leaves (Huber 1927), with special consideration of the time of exposure of the leaf after cutting (Halevy 1956).

Preliminary studies of transpiration of detached gladiolus leaves in successive minutes disclosed that no significant change in water loss occurs till the 4th minute after plucking; later on, there was a significant decline in transpiration rates. Therefore we were careful to accomplish the initial and the final weighing within 3 minutes after cutting. The medium portion of the third and the fourth leaf from bottom was used in 4 replicates.

2. *Stomatal opening* measurements were carried out by the infiltration method, using series of 11 liquids developed by us for gladiolus leaves, in analogy to the mixtures of Schorn (1929). The liquids were mixtures of odorless kerosene ("white oil"), innocuous to plants, and paraffin oil, of predetermined viscosity. They differed

rom each other by steps of 10% by volume; so that liquid No. 1 was pure kerosene—aving the lowest viscosity, liquid No. 2 was a mixture of 90% kerosene and 10% paraffin oil, and No. 11 was pure paraffin oil—with the highest viscosity.

The infiltration liquids were applied to the medium portion of intact leaves using nixtures of decreasing viscosity. The number of the first liquid infiltrating within seconds, was considered as indicating the degree of stomatal aperture of this eaf. Each recorded figure is the average of measurements for 10 leaves.

The exact correlation of the degrees of infiltration under average condition of emperature, with actual stomatal aperture, was evaluated by direct microscopic xaminations (Stålfelt 1929). (Table I).

The stomatal apertures of all five leaves were determined.

TABLE I

The stomatal aperture corresponding to infiltration grades

First mixture producing infiltration in 5 seconds	Average width of stomates (μ)
1	0–1
2	0–2
3	1–3
4	2–3
5	3–4
6	4–6
7	6–7
8	6–8
9	6–8
10	7–9
11	8–9

3. *Water content* was determined in the medium portion of each leaf (4 replicates). The water content of the third and the 4th leaf was determined in the same specinens examined for transpiration. The initial weighing was considered as "fresh veight".

4. *Water saturation deficit* (WSD) determinations were carried out in the medium portion of the 3rd and the 4th leaves (4 replicates) by Stocker's (1929) method, lightly modified and adjusted for gladiolus leaves.

5. *Osmotic values* (OV) were measured by the cryoscopic method, adjusted by Currier (1944) for small amounts of tissue sap. Only third leaves were investigated. Each figure is an average of 4 samples of sap expressed from five whole leaves.

6. *Temperature* and *relative humidity* were determined by a ventilated psychroneter. Instead of relative humidity, relative saturation deficit of the air is indicated n the figure.

7. *Evaporation* was measured by means of a Piche evaporimeter suspended near he plants. The evaporimeter was protected from sun radiation by a special helter, used by the Israel Meteorological Service.

RESULTS AND DISCUSSION

The results are summarized in Figure 1.

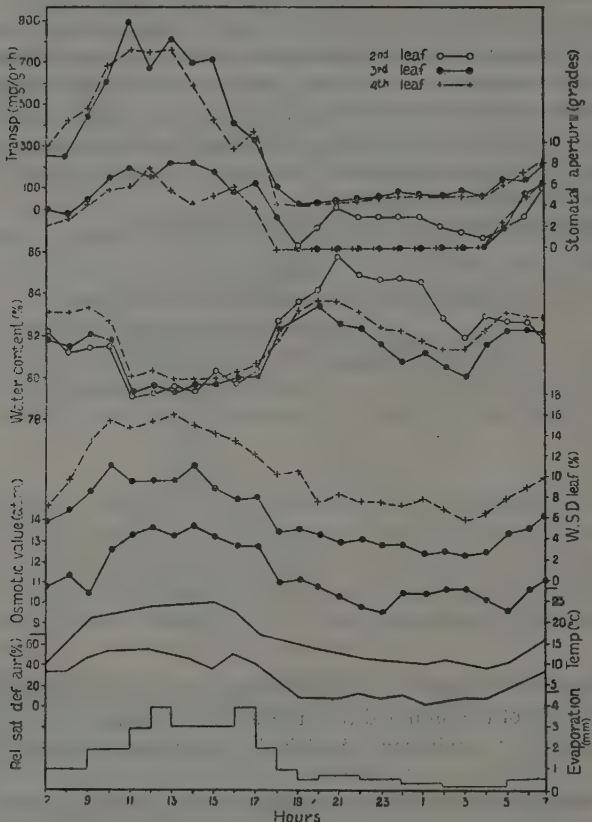

Figure 1

Diurnal curves of fluctuations in water balance factors of gladiolus leaves, in relation to meteorological data (April 23, 1957).

1. *Transpiration.* The transpiration curves of the 3rd and the 4th leaves are similar. These are one-peak curves. Transpiration rose gradually from sunrise, but mainly from 9 p.m., and attained its peak about 11 h., coinciding with the peak in the main meteorological factors. The high water loss continued at the same level for about 5 hours. The decline in transpiration started at 16 h., before the main decrease in light intensity and evaporation; the reason seemed to be the leaf water deficit, which apparently produced primarily a reduction in stomatal aperture. The decrease in transpiration and stomatal aperture started earlier in the upper – 4th leaf, since its

WSD was considerably higher than in the 3rd leaf. During the night, transpiration was very low; 0 to 100 mg/gm.h., about 1/10 of the rates at noon.

2. *Stomatal opening*. Gladiolus stomates are very sensitive to light and darkness. They open immediately after sunrise and close completely when darkness approaches. They are sensitive to water deficit, too. The higher WSD in the 4th leaf apparently caused a marked reduction of the stomatal aperture in the afternoon. A close relation was found between transpiration rates and the degree of stomatal opening in the lower apertures, till mixture 6 (5μ), A significant positive correlation between the stomatal aperture till 4μ and the rate of transpiration has been established (Halevy 1958). No such correlation was found in the higher degrees of stomatal opening.

An interesting phenomenon is the night opening of the stomata on the lower two leaves (The diagram shows only a second leaf curve). The stomata of these leaves closed in the evening as usual. After they had been closed for few hours, they reopened at about 20 h. This opening lasted till 2 a.m. Later followed a reduction in aperture till 4 h., and finally the usual opening at sunrise.

It seems that this surprising opening at night is connected with the rising water content of these leaves, reaching its maximum at 21 h. During this time the water content of the lower leaves was 2 to 4% higher than that of the upper leaves. This difference is highly significant statistically. Only after midnight, when the water content decreased, did the stomata close again.

We did not find any indication of a similar phenomenon in the literature. Open stomata at night have indeed been found by Loftfield (1921). But they did not close from sunset to sunrise at all, while in our case they opened after they had been closed early in the night. Even Stålfelt (1932, 1955, 1957), in his classic investigations on the mechanism of stomatal opening, did not mention such a case. Nor could we find an explanation of this stomatal opening, since it cannot be explained by either the hydroactive, or the hydropassive mechanism. Recently, Prof. Stålfelt (1958) informed me that occasionally he had met with a similar unusual night opening of stomata.

3. *Water content* was determined in all five leaves, but only data of leaves 2, 3, and 4 are presented in the diagram.

The water content of all leaves decreased at about 11 h., and remained at low levels till sunset. Thereafter the stomata closed and transpiration stopped – so the water content rose rapidly to a maximum which was attained at about 20 h. These changes are matched by antibatic changes of the WSD as well. From about midnight the water content began to decrease to a minimum, attained two hours before sunrise, and then again increased till the drop before noon. The decrease in water content after midnight was found statistically highly significant.

The diurnal water content of the lower leaves 1 and 2 was similar to that of the other leaves. But at night their rates were remarkably and significantly higher. They persisted at their high level till 2 a.m.; only then a decrease lasting for 2–3 hours took place.

The reduction of water content in the later part of the night seems to be a widespread phenomenon, since it was found also by Stanescu (1936), Portsmouth (1937)

Kramer (1937) and Wilson et al. (1953). Meyer and Anderson (1952) explained this decline by suggesting that "during the early morning hours the leaves lose water by translocation to other organs of the plant." Contrary to this theory, Wilson et al. (1953) suggest that this decrease in leaf moisture is merely a reflection of an increase in its dry weight. They did not give any proof of their theory.

Our experiment brings out some facts in favour of the latter explanation. If the leaves lose water after midnight, not only should their water content decrease, but their WSD would rise. That did not happen. During all the time that the water content decreased, there was a slight but constant *decrease* in WSD. Furthermore at the same time there was an increase in osmotic values of the same leaf, even though the transpiration was almost zero. Apparently the cause for this decrease in water content is not water movement from the leaves, but *dry weight* increase, by translocation of dry matter to the leaves and/or by organic synthesis in them. It seems that this "dry matter" is osmotically active material.

4. *Water saturation deficit*. The most striking fact in the WSD curve is the higher deficit of the 4th leaf throughout the 24-hour period. The general progression of WSD is similar in both leaves. The deficit rose rapidly in the morning, attaining its maximum at 10 a.m. The high deficit persisted for about 6 hours, with a slight decrease at midday, coinciding with the partial decrease in stomatal aperture and transpiration. Later in the afternoon, when transpiration rates began to drop – the WSD decreased gradually and continuously during the entire night, until the minimum was reached about two hours before sunrise. Soon afterwards the deficit increased again.

The rise of WSD during the daylight hours is caused by the lag in the rate of absorption as compared with the rate of transpiration (Kramer 1937). During the night, absorption exceeds transpiration. But in spite of the very high soil moisture content and very low transpiration rates, there was always a deficit of 2.5–6.1%. This is in line with the results of Stocker (1929) who stated that plants planted in soil could never attain full saturation, even if their tops are in a saturated atmosphere.

Weatherley (1951) discussed the reasons for the water deficit before sunrise and suggests two hypotheses to explain it: a) The increasing resistance of the roots to absorption of water, when transpiration is very low. b) The forces retaining the water in the soil which are higher in the root zone than outside it, and might, in the author's opinion, prevent the roots from absorbing enough water to remove the deficit.

Thus both hypotheses concentrate on the inability of the roots to absorb water, for reasons immanent in the root or in the surrounding soil. Weatherley (1951) does not discuss the possible resistance to water movement within the plant and especially the water conductive vessels and the leaf tissue. The results of the present experiment indicate that the resistance to translocation of water within the plant is, at least in certain cases, as important in producing such deficits, as the difficulties in water absorption by the roots. A highly significant difference of about 5% was found between the WSD of the 4th and the 3rd leaf, during most hours, while the

total difference between the minimal and the maximal figures of each individual leaf during the 24-hour period did not exceed 8.5–10%. The lowest deficit of the 3rd leaf, at 3. a.m., was 2.5%. This deficit was probably produced by difficulties in root absorption and translocation. The WSD of the 4th leaf at the same time was 6.1%, i.e. 3.6% higher than that of the 3rd leaf. This additional deficit was apparently caused by the additional resistance to water movement between the lower and the upper leaf and probably by differences in DPD. This conception is strengthened by the results of other experiments (Halevy 1958) showing that in gladiolus plants growing in a saturated soil, there is a significant increase in WSD from the 3rd leaf upward. The deficit of the upper part of most leaves was also significantly higher than that of the lower part of the same leaf.

5. *Osmotic values* rose rapidly in the morning from 10.3 atm. at 9 h. to 13.1 atm. at 11 h. The same level of OV existed till late in the afternoon. Afterwards a gradual decrease took place until midnight, followed by a slight increase till 3 p.m., and another decrease till sunrise.

Two causes may produce an increase in osmotic values; a decrease in water content, and an increase in the quantity of osmotically active substances. During the daylight hours significant correlations were found between OV and WSD ($r = \pm 0.63$), and water content ($r = -0.71$). Apparently, during those hours, the water balance fluctuations are the main cause of the variations in osmotic values. No such correlation was found during the night hours. It seems that the variations in rates during these hours are mainly governed by changes in the content of osmotically active substances, as suggested above.

The practical purpose of this investigation was to find the right hour of the day for testing the changes caused in water balance by soil moisture stress, as a basis for experiments attempting to find a physiological test suitable as an indication for irrigation. The results of this experiment indicate that, when soil moisture is adequate, no major changes in water balance of gladiolus leaves occur until 9 a.m. Therefore we concluded that the most suitable time for these tests should be 8 a.m., i.e. about 3 hours after sunrise. This was the same hour that Oppenheimer and Elze (1941) chose to test citrus trees for irrigation requirement.

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REFERENCES

Beck, W. A., 1931, Variations in Og of plant tissues, *Plant Phys.*, **6**, 315–323.
Burgerstein, A., 1925, Die Transpiration der Pflanzen I–III, Fischer, Jena.
Compton, C., 1936, Water deficit in citrus, *Proc. Amer. Soc. Hort. Sci.*, **34**, 91–95.
Crafts, A. S., Currier, H. B. and Stocking, C. R., 1949, Water in the physiology of plants, *Chronica Botanica*, Waltham, Mass., 420 pp.

Currier, H. B., 1944, Cryoscopy of small amounts of expressed tissue sap, *Plant Phys.*, **19**, 544–590.

Halevy, A., 1956, Orange leaf transpiration under orchard conditions, IV. A contribution to the methodology of transpiration measurements in citrus leaves, *Bull. Res. Counc. of Israel*, **5D** 155–164.

Halevy, A. H. 1958, Studies on water relations of gladiolus (in Hebrew). Ph.D. thesis, Hebrew University, Jerusalem.

Herrick, E. H., 1933, Seasonal and diurnal variations in the osmotic values and suction tension values in the aerial portions of *Ambrosia triflda*, *Amer. J. Bot.*, **20**, 18–34.

Huber, B., 1927, Zur Methodik der Transpirationsbestimmung am Standort, *Ber. Dtsch. Bot. Ges.*, **45**, 611–618.

Kramer, P. J., 1937, The relation between rate of transpiration and rate of absorption of water in plants, *Amer. J. Bot.*, **24**, 10–15.

Loftfield, J. V. G., 1921, *The behaviour of stomata*, Carnegie Inst. Wash. Pub. 314, 1–104.

Marsh, F. L., 1940, Water content and osmotic pressure of certain prairie plants in relation to environment, *Nebraska Univ. Stud.*, **40**, 3–44.

Maximov, N. A., 1929, The plant in relation to water (English edition by Yapp), London, 451 pp.

Meyer, B. S. and Anderson, D. B., 1952, *Plant Physiology* (2nd edition). Van Nostrand Co., Princeton, 784 pp.

Morello, G., 1953, Transpiracion y balance de àgua de la banaera en las condiciones de la ciudad de Sao Paulo, *Bul. Fac. Fil. Cien. Univ. Sao Paulo, Bot.*, **10**.

Nitzan, J., 1954, A comparison of two methods for determinations of water saturation deficit (in Hebrew), M.Sc. thesis, Fac. of Agric., Hebrew University, Rehovoth.

Oppenheimer, H. R. and Elze, D. L., 1941, Irrigation of citrus trees according to physiological indicators, *Pal. J. Bot. Reh. Ser.*, **4**, 20–46.

Oppenheimer, H. R. and Mendel, K., 1939, Orange leaf transpiration under orchard conditions, I. *Pal. J. Bot. Reh. Ser.*, **2**, 171–250.

Portsmouth, G. B., 1937, Variations in the leaves of cotton plants grown under irrigation in the Sudan Gezira, *Ann. Bot. London* (*N.S.*), **1**, 277–291.

Runyon, E. H., 1936, Ratio of water content to dry weight in leaves of the creosote bush, *Bot. Gaz.*, **97**, 518–553.

Schorn, M., 1929, Untersuchungen über die Verwendbarkeit der Alkoholfixierungs- und Infiltrationsmethode zur Messung der Spaltoeffnungsweiten, *Jahrbuch Wiss. Bot.*, **71**, 783–840.

Shmueli, E., 1953, Irrigation studies in the Jordan Valley I. Physiological activity of the banana in relation to soil moisture, *Bull. Res. Counc. of Israel*, **3**, 228–244.

Stalfelt, M. G., 1929, Neuere Methoden zur Ermittlung des Oeffnungszustandes der Stomata, *Handb. der Biolog. Arbeitsmethoden*, 167–192.

Stalfelt, M. G., 1932, Der stomatäre Regulator in der pflanzlichen Transpiration, *Planta*, **17**, 22–85.

Stalfelt, M. G., 1955, The stomata as a hydrophotic regulator of the water deficit of the plant, *Physiol. Plant.*, **8**, 572–593.

Stalfelt, M. G., 1956, Die stomatäre Transpiration und die Physiologie der Spaltoeffnungen, *Handb. d. Pflanzenphys., Berlin*, **3**, 351–426.

Stalfelt, M. G., 1957, The water output of the guard cells of the stomata, *Physiol. Plant.*, **10**, 752–773.

Stalfelt, M. G., 1958, A private letter to the author.

Stanescu, P. P., 1936, Daily variations in products of photosynthesis, water content and acidity of leaves towards end of vegetative period, *Amer. J. Bot.*, **23**, 374–379.

Stocker, O., 1929, Das Wasserdefizit von Gefaesspflanzen in verschiedenen Klimazonen, *Planta*, **7**, 382–387.

Stocker, O., 1956, Die Abhängigkeit der Transpiration von den Umweltfaktoren, *Handb. d. Pflanzenphys. Berlin*, **3**, 436–488.

Stoddart, L. A., 1953, Osmotic pressure and water content of prairie plants, *Plant Phys.*, **10**, 661–680.

Weatherley, P. E., 1951, Studies in the water relations of the cotton plant. II: Diurnal and seasonal variations in relative turgidity and environmental factors, *New Phytol.*, **50**, 36–51.

Wilson, C. C., Boggess, W. R. and Kramer, P. J., 1953, Diurnal fluctuations in the moisture content of some herbaceous plants, *Amer. J. Bot.*, **40**, 97–100.

TRANSLOCATION OF SALTS IN *VALLISNERIA* LEAVES

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ABSTRACT

The problem is studied, whether labelled cations of salts, absorbed by a part of a *Vallisneria* leaflength, are exchanged during their translocation to the adjoining free zone, when this zone is immersed in a salt solution with nonlabelled cations.

Exchange has been found to be small. When the free zone is placed in a KCl solution it accumulates K and Cl ions. As a consequence the normal translocation of the labelled cations from the absorbing to the free zone is dammed up.

The leaves of *Vallisneria spiralis* have cells of regular shape and feebly developed bundles without xylem vessels, but containing sieve tubes. This object has permitted the use of a method which compares the processes in the absorbing part of a leaf-length with those in adjoining parts not in direct contact with the outer solution. This method has enabled us to distinguish a process introducing the salt into the cytoplasm from processes of accumulation in the cytoplasm, secretion into the vacuoles and translocation through the symplasm (Arisz 1958). A scheme has been given in that paper of the active processes in the root.

Figure 1 gives a slightly altered scheme of the absorption and translocation processes which can be used as a basis for the present discussions.

At least four processes can be distinguished:

1. A passive penetration of ions into the plasmatic free space (Briggs and Robertson 1957), here designated as the peripheral plasm. This can be a simultaneous diffusion of anions and cations or an exchange mostly of cations present in the Donnan space for cations of the external solution. The apparent free space in *Vallisneria* leaves (wall free space, plasmatic free space and adherent water) amounts to 5–7% of the cell volume, as was found by Kylin and confirmed by Winter (unpublished results).

2. Active absorption of anions into the central plasm. The anions which have passed the peripheral cytoplasm are not given off when the leaf is brought into water, neither are they exchanged for anions in the outer solution. Whether these anions remain free or are bound is not known.

3. Active secretion of cations and anions from the central plasm into the vacuoles.

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4. Translocation of ions in the symplasm.

Since *Vallisneria* leaves are always surrounded by an external solution a translocation in that part of the symplasm, which can be considered as free space, does not play a role in the normal movement of salt ions along the leaf. The cellular translocation has to take place in the central plasm.

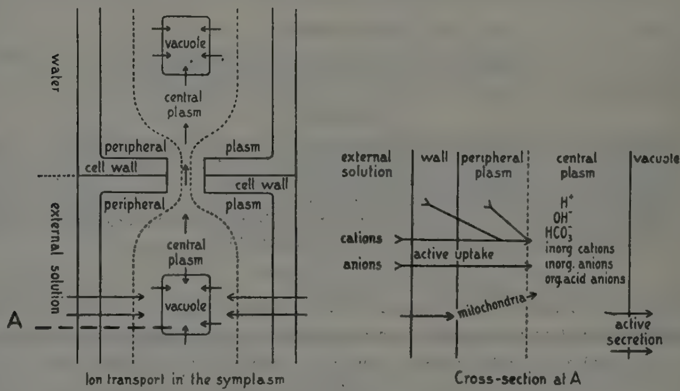

Figure 1

Schematic representation of the uptake processes in *Vallisneria* leaves. Two adjoining cells are interconnected by a plasmodesm. The ions are absorbed in the peripheral plasm by a passive and in the central plasm by an active process. From the central plasm the ions are actively secreted into the vacuoles or translocated through the plasmodesmata to contiguous cells. The right part of the figure represents a cross section through one of the cells at A.

The symplasm theory has been proved by demonstrating that translocation into the "free zones", i.e. those zones not in direct contact with the outer solution, is continued when their uptake out of the free space is inhibited by local application of cyanide, arsenate or uranyl nitrate. In that condition the passage from cell to cell has to be via the cytoplasm of the cells, interconnected by plasmodesmata in which the central plasm forms a continuous pathway (Arisz 1953, 1958).

The scheme is only a simplification of the processes involved. The ions introduced in the central plasm, anions such as chloride, phosphate, sulphate and bicarbonate, and cations such as potassium, rubidium or calcium, may participate in metabolic reactions and it depends on their character whether they will remain free or will be bound to plasmatic structures or involved in metabolic transformations. The character of the ions does not only decide their conduct in the cytoplasm but also whether they will be accumulated into the vacuoles. Thus potassium and rubidium ions seem to have a greater affinity for being secreted into the vacuoles than calcium ions. An analogous difference may exist between chloride and phosphate ions;

trate ions are probably not secreted into the vacuoles nor accumulated in the proto-asm. Whether they penetrate into the cytoplasm is not sure. Bicarbonate ions osorbed in the cytoplasm will probably be involved in metabolism and produce rganic acid anions by carboxylation of pyruvate. These organic acid anions in the ntral plasm can be secreted into the vacuoles. These considerations keep us from pecting that a scheme will be generally valid and applicable to all kinds of ions.

In our former scheme we followed the assumption of Lundegardh that the anions e actively translocated to the central plasm while the cations freely diffuse in the ytoplasm. In respect of the anions there is unanimity that after uptake they cannot e exchanged any more.

It is a fundamental problem whether the cations in the central plasm are exchange-ble for ions in the external solution. Before their "active uptake" some cations in e leaf cells can be exchanged for ions from the external solution, but after their ptake exchange has become impossible. This process of ion fixation can be studied y bringing the tissue first into contact with the external solution and tracing the mount of ions still exchangeable after the tissue has been for different periods in ater. The process of cation fixation is then continued (Epstein, and Leggett). The nature f this 'fixation process' is unknown. It can be the passage of a barrier layer or the nding of the cations to stationary structures in the protoplasm or to movable rriers. If the active process is the passage of a barrier it can be the passing either f the peripheral cytoplasm or of the tonoplast. Having passed the barrier the ions ay be free or bound. (cf. Winter. unpublished results).

The problem whether during their translocation in the symplasm cations are changeable for cations from the external solution can be studied by observing the anslocation of labelled cations, e.g. rubidium ions, while the free part of the leaf brought into solutions of different potassium salts. By immersing only a 2.5 cm one of a leaflength of *Vallisneria* (length 7.5 cm) in the external solution an absorbing one is obtained in direct contact with the external solution and a second and a third one, here called the free zones, to which the salt is transferred via the symplasm. he translocation of labelled rubidium and chloride ions from the absorbing zone the free zones has been investigated while the free part of the leaf was either in ater or in a solution of potassium chloride or potassium nitrate. In the salt solutions n exchange can be expected of exchangeable rubidium ions in process of being anslocated in the central plasm for potassium ions of the external solution.

First we have to discuss the more simple case, the absorption of potassium chloride r potassium nitrate by a leaflength of 2.5 cm. The substances have been chosen ecause KCl and RbCl are typical for salts of which the cat- and anions are absorbed nearly equal amounts, while potassium nitrate and potassium phosphate are ibstances of which the cation uptake is much larger than the anion uptake. If icarbonates are also present in the solution a surplus amount of cations can be osorbed simultaneously with bicarbonate ions (Hurd 1958). If bicarbonate

ions are taken away from the external solutions and carbon dioxide is eliminate the cation uptake is still much larger than the phosphate or nitrate uptake. It i found that processes take place resulting in an excretion of calcium and magnesiu ions. The exchange of K for hydrogen ions from the tissue, if present, is rather small which appears from the small increase of the hydrogen ion concentration of the ex ternal solutions (cf. Jacobson 1955).

It can be concluded that in ordinary absorption experiments both ions are activel absorbed in about equal amounts from a KCl solution, while from KNO_3 solution nitrate ions are not absorbed at all and potassium in a lower amount than from chloride solution. We now revert to the translocation experiments.

In a first experiment the absorbing zone of a leaflength of 7.5 cm was brought i contact with a 2mM labelled rubidium chloride solution for a period of 24 hours Rubidium chloride was absorbed and a small amount was translocated to the fre zones. After this period the free zones of the leaf were placed either in water or i a 2 mM solution of potassium chloride or potassium nitrate for 4 hours. Durin this period there was no appreciable loss of rubidium in process of being translocate in the tissue to the external solution. The lack of exchange may have been due not to a non-exchangeability of the moving cations, but to the very low concentration of the exchangeable cations in the central plasm after 24 hours uptake, since it ca be expected that as a result of continuous secretion into the vacuoles the ion con centration in the plasm will be brought to a rather low level. It seemed appropriat to repeat the experiment with a shorter uptake and a longer exchange period. Th absorption period was diminished to 4 hours and the exchange period prolonge to 24 hours. During both periods the absorbing zone was in contact with 2 mM labelled RbCl solution. During the absorption period the free part was in water an during the exchange period either in water (A) or in KCl (B) or KNO_3 (C). Table

TABLE I

Distribution of labelled Rb in absorbing and free zones of* Vallisneria *leaves at conclusion o "exchange period" in water or K salts*

(All leaves were first pretreated in water in the light for 24 hr. The absorbing zones were then trans ferred to 2 mM labelled Rb*Cl for 4 hr., while the free zones remained in H_2O. During the subsequen 24 hr. the absorbing zones remained in 2mM Rb*Cl while the free zones were treated as indicated i the Table. Each sample comprised 8 leaflengths 7.5 cm long. The leaves were continuously expose to light at 25°C)

	Solution surrounding free zones during final 24 hr.	Rb* detected in leaf (μM)		
		Absorbing zone	Free zones 1	Free zones 2
A	H_2O	6.9	1.5	0.9
B	2 mM KCl	8.9	0.3	0.0
C	2 mM KNO_3	6.3	0.8	0.1

gives the amount of labelled rubidium ions present in the three zones of the leaflength at the end of the exchange period. The quantity of labelled rubidium ions given off to the external solution in contact with the free zones was small in comparison with the amount translocated in the tissue in the case of (B). It was somewhat larger in the case of (C). Since the plant material was not of superior quality the values obtained for the loss of rubidium to the outer solution cannot be considered as sufficiently reliable.

It is a striking fact that in the series with the free zones in KCl solution the rubidium absorbed in the absorbing zone was not translocated in the normal way to the free zones. It was dammed up in the absorbing zone. This was caused by the simultaneous active uptake of KCl by the free zones, which inhibited the normal flow of rubidium in the symplasm of the leaf to the free zones by diminishing the concentration gradient for rubidium ions because potassium and rubidium ions are competitive in absorption processes. The total amout of rubidium ions in the three zones of A and B was the same. There was only a difference in their partition over these zones.

In (C), where the free zones were in KNO, there was no question of such a damming up of the rubidium ions in the absorbing zone. The amount of rubidium translocated to the free zones was larger than in B, but smaller than in A. The difference could be the result of the absorption of chloride ions in B, while in C no nitrate ions were absorbed, but there was also a difference in the amount of potassium ions absorbed in B and C. The total amount of rubidium ions in the three zones was in this case smaller than in A and B. The data are not sufficient to exclude an exchange of rubidium ions for potassium ions from the potassium nitrate solution.

In the hope of getting more information about this problem the exchange was also studied in another way. After 4 hours absorption in KCl, with the free zones in water, the free zones of the leaflengths were either allowed to remain in water (D), or were transferred to labelled rubidium chloride (E) or to labelled rubidium nitrate solution (F). This permits us to study the absorption of labelled rubidium by the free part of the leaf during the translocation of potassium chloride from the absorbing to the free zones. At the same time the increase in chloride was estimated by the Volhard method.

In labelled rubidium chloride a strong uptake of rubidium and a still larger one of chloride by the free zones was observed (Table II). This result could have been expected. We leave out of the discussion here the stronger accumulation in the third zone as compared with the second zone. It is the strong accumulation of rubidium chloride by the free zones which causes the damming up effect found in the preceding experiment.

The conclusion to be drawn from these experiments is that bringing the free part of a translocating leaflength in contact with potassium chloride solution produces a damming up of the salt absorbed in the absorbing zone. The labelled rubidium ions are obstructed in their flow to the free zones (Table I) since the potassium ions

abscrbed in the plasm diminish the concentration gradient for potassium and rubidium ions in the symplasm.

TABLE II

The absorption of labelled Rb by the free zones of* Vallisneria *leaves during the translocation of KCl from the absorbing to the free zones*

(All leaves were first pretreated in water in the light for 24 hr. The absorbing zones were then transferred to 2 mM KCl for 4 hr. while the free zones remained in H_2O. During the subsequent 24 hr. the absorbing zones remained in 2 mM KCl while the free zones were treated as indicated in the Table)

	Solution surrounding free zones during final 24 hr.	Rb* detected in leaf (μM)			Increase in Cl in leaf (μM)		
		Absorbing zone	Free zones 1	Free zones 2	Absorbing zone	Free zones 1	Free zones 2
D	H_2O				8.4	0.9	0.3
E	2 mM Rb*Cl	0.4	10.3	11.8	10.6	11.6	14.2
F	2 mM Rb^*NO_3	0.1	2.6	3.9	7.6	0.8	0.6

In the experiment where the free zones were in potassium chloride solution an exchange of moving labelled rubidium ions for potassium ions from the external solution could not be detected. This may have been due to the active uptake of potassium chloride which effected the damming up of the rubidium ions in the absorbing zone.

When the free zones were in potassium nitrate solution (Table IC) the uptake and translocation of the labelled rubidium ions was relatively remarkably low. The total difference with A amounts to 2.1 μM Rb, that is to 23%. Since the amount of rubidium ions detected in the external solution after the experiment was never higher than about 0.1 μM it is not likely that this considerable difference was due to ion exchange. Further experiments will be needed to elucidate this effect.

This publication is dedicated to Professor H. Oppenheimer on his 60th birthday.

REFERENCES

Arisz, W. H., 1953, *Acta bot. neerl.*, **1**, 506–515.

Arisz, W. H., 1956, *Protoplasma*, **46**, 5–62.

Arisz, W. H., 1958, *Acta bot. neerl.*, **7**, 1–32.

Briggs, G. E. and Robertson, R. N., 1957, *Annual Rev. Plant Physiology*, **8**, 11–29.

Epstein, E. and Leggett, J. E., 1954, *Amer. J. Bot.*, **41**, 785–91.

Hurd, R G., 1958, *J. exp. Bot.*, **9**, 159–175.

Jacobson, L., 1955, *Plant Physiology*, **30**, 264–269.

Winter, H., Unpublished results.

NOTE SUR LA VEGETATION HALOPHILE AU SAHARA OCCIDENTAL

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RESUME

La végétation halophile se rencontre au Sahara occidental dans trois types de station: 1) Les vallées colonisées en général par la savane – *Acacia-Panicum* que la présence de sel modifie peu à peu en lande à Salsolacées ou à *Limoniastrum ifniense*, 2) La steppe à *Arthrophyton scoparium* beaucoup plus pauvre qui évolue elle aussi avec le salant en steppe à *Zygophyllum*, *Traganum*, *Salsola*, *Atriplex*. 3) Les Sebkhas dont la plus remarquable, celle de Timimoun est occupée par l'association à *Randonia africana* et *Henophylon deserti*.

INTRODUCTION

.a surface occupée par les terrains salés au Sahara occidental est relativement aible et, souvent, un ensablement plus ou moins important, parvient à les masquer.

Cependent, la végétation spontanée, malgré un très faible coefficient de recouvrement, indique toujours parfaitement la salinité des sols, des nappes superficielles et les eaux phréatiques.

Divers groupements végétaux, par leur constitution floristique, permettent de caractériser les différents degrés de salure avec une précision égale à celle obtenue dans les reconnaissances phytosociologiques effectuées sur les solontchaks humides des plaines sub-littorales du Tell ou des Hautes plaines steppiques.

Toutefois, les observations doivent porter essentiellement sur les *espèces pérennantes*, qui sont les mieux adaptées à ce facteur édapho-climatique. Très peu d'espèces annuelles, en effet, caractérisent strictement sous le climat désertique les zones salées et surtout hypersalées.

Au Sahara, en effet, de faibles précipitations entraînent bien souvent l'apparition de nombreuses espèces annuelles à cycle évolutif extrêmement bref: "l'acheb" dont le développement est lié aux couches toutes superficielles de sol et dont la valeur phytosociologique n'est cependant pas nulle au Sahara central et méridional en particulier, mais qui, cependant, dans la région qui nous intéresse ici ne jouent qu'un rôle fort discret dans les groupements halophiles.

Les groupements végétaux halophiles du Sahara occidental tous caractérisés par l'extrême pauvreté de la flore, sont localisés:

— dans les vallées irrégulièrement inondées par des crues plûs ou moins éphémères.
— sur le pourtour des sebkhas.
— dans quelques dépressions ou dayas.

LA VEGETATION HALOPHILE DES VALLEES

L'ensablement des lits d'oueds, qui entaillent plus ou moins profondément la hamada, peut être parfois considérable et les dunes, relativement fixées par la végétation spontanée, atteindre 10 à 15 mètres de hauteur (Aouinet Legra).

Malgré ces conditions défavorables à première vue, la richesse floristique augmente toujours avec l'importance de l'alluvionnement.

Toutefois, la végétation annuelle, qui profite des précipitations pluvieuses et plus rarement des eaux de crues, a le plus souvent un cycle évolutif très bref. Les plantes se hâtent de pousser, fleurir et fructifier pour disparaître au moment où sont épuisées les réserves en eau de l'horizon superficiel du sol fouillé par leurs racines.

Il n'en est pas de même pour les espèces vivaces, à enracinement profond, atteignant les couches inférieures du sol qui conservent plus longuement une certaine humidité ou sont même traversées en permanence par l'inféroflux de l'oued.

Aussi la plupart des vallées sont-elles matérialisées en toutes saisons par un cordon continu de touffes, de buissons d'arbustes ou même d'arbres. Le tracé du moindre petit oued, dont le lit n'est emprunté que très irrégulièrement par les eaux de ruissellement, se trouve ainsi mis en évidence. Ce qui donne un aspect très particulier à la physionomie végétale de ces régions.

Les espèces pérennantes sont toujours dominantes et la présence du salant détermine l'apparition d'espèces spécialisées appartenant surtout aux familles des Chénopodiacées et des Zygophyllacées.

La savane à *Acacia-Panicum* (Ch. Sauvage 1946) ou savane désertique à gommiers— association à *Acacia raddiana* et *Panicum turgidum* (M. Guinochet et P. Quezel 1954) ou pseudo-steppe arborée à *Acacia raddiana* (Ph. Guinet 1954) est le groupement végétal caractéristique des vallées ou des lits d'oueds du Sahara occidental, et c'est à partir de celui-ci que dans divers cas des groupements très affinés accusent la salure au moins des couches profondes du sol.

Divers groupements peuvent être individualisés:

1. Le groupement type, très riche en espèces, est bien représenté dans les monts d'Ougarta et dans la partie orientale du Sahara espagnol.

D'après Guinet (1954), qui l'a étudié dans les vallées des monts d'Ougarta, le nombre des espèces s'élève à:

20 dans le lit de l'Oued Oum Bou Raï
24 dans l'Oued Farès
28 dans l'Oued el Abiod
13 à Nif el Kroufi
18 à Foum el Moukahla

Dans le Sahara espagnol, 39 espèces ont été déterminées dans la vallée de l'Oued Tifariti (alluvions sablo-limoneuses, souvent très grossières) où la végétation est la mieux développée. Ce sont:

Acacia raddiana Savi
Pergularia tomentosa L.
Cleome arabica L. (un sujet)
Peganum harmala L. (un sujet)
Zizyphus lotus (L.) Lamk.
Linaria aegyptiaca (L.) Dum. Cours. ssp. *battandieri* Maire
Enneapogon brachystachyus (Haub. et Spach) Stapf
Ephedra alata Dec. ssp. *alenda* (Stapf) Trabut
Periploca laevigata Ait.
Gymnocarpus decander Forsk.
Glossonema boveanum Dec. var. *gautieri* Batt. et Trab.
Centaurea pungens Pomel
Anvillea radiata Coss. et Dur. var. *genuina* Maire
Aristida ciliata Desf. var. *genuina* Trin. et Rupr.
Cenchrus ciliaris L. var. *genuinus* (Leeke) Maire et Weiller
Cymbopogon schoenanthus (L.) Spreng. ssp. *laniger* (Hook.) Maire et Weiller
Aristida obtusa Del.
Bubonium graveolens (Forsk.) Maire
Trichodesma calcaratum Coss.
Nitraria retusa (Forsk.) Asch.
Paronychia arabica (L.) DC. ssp. *cossoniana* (J. Gay) Maire et Weiller
Heliotropium undulatum Vahl ssp. *eu-undulatum* Maire var. *suffruticescens* (Pomel) Maire
Lycium intricatum Boiss.
Arthrophytum scoparium (Pomel) Iljin
Euphorbia calyptrata Coss. et Dur. var. *involucrata* Batt.
Atriplex halimus L.
Panicum turgidum Forsk.
Farsetia hamiltonii Royle
Lotus jolyi Batt. ssp. *battandieri* Maire var. *asperulus* Maire
Andrachne telephioides L.
Caylusea hexagyna (Forsk.) Maire var. *rigida* (Mull. Arg.) Maire
Convolvulus trabutianus Schweinf. et Muschl.
Echinops spinosus L. ssp. *bovei* (Boiss.) Murb. var. *pallens* Maire
Suaeda monodiana Maire
Rhus tripartitum (Ucria) R. et Sch. var. *typicum* Maire
Launaea arborescens (Batt.) Maire
Nucularia perrini Batt.
Salsola foetida Del. var. *eu-foetida* Maire

Une composition floristique semblable a été observée dans les relevés effectués dans les vallées des oueds Tamudchad, Ben Aamera, Um Chemel, etc. tous situés dans la partie Est du Sahara espagnol.

Il faut toutefois remarquer qu'il s'agit ici d'un relevé global effectué par un de nous (S) à la faveur d'une trop brève halte, et dans lequel s'imbriquent certainement en zones successives, comme c'est toujours le cas dans les lits d'oueds du Sahara, plusieurs groupements végétaux: lit d'oued à *Acacia*, rocailles marginales à *Aristida*, *Trichodesma*, *Echinops*, *Nucularia*, *Launaea*, etc. en particulier. Dans ces cas la salure des couches profondes est toujours très faible, certainement inférieure à 2 g/kg. Seule, l'indique la présence de *Nitraria retusa*, *Lycium intricatum*, *Suaeda monodiana* et *Salsola foetida*.

2. Bien souvent le cortège floristique est fortement appauvri. Tel est le cas dans la région de Tindouf où les alluvionnements sont toujours peu importants. Le cordon végétal ne renferme plus que six espèces:

Acacia raddiana Savi
Pituranthos battandieri Maire ssp. *abbreviatus* Maire
Convolvulus supinus Coss. et Kral. var. *astrichogynus* Maire et Wilczek
Morettia canescens Boiss. var. *typica* Maire = *eu-canescens* Maire
Linaria aegyptiaca (L.) Dum. Cours. ssp. *battandieri* Maire var. *typica* Maire.
Cymbopogon schoenanthus (L.) Spreng. ssp. *laniger* (Hook.) Maire et Weiller

3. Enfin, dans les vallées de la Hamada du Guir, ce cortège est encore plus réduit, et limité à quelques touffes très disjointes de *Panicum*, *Pituranthos*, *Convolvulus*, *Cymbopogon* et à quelques pieds souffreteux d'*Acacia*.

Dans ce groupement végétal, spécifique des lits d'Oueds du Sahara occidental, le salant se manifeste selon le processus suivant, particulièrement apparent dans les vallées du Sahara espagnol où l'action humaine est pratiquement nulle:

Diminution de la taille des arbres (*Acacia raddiana*, *Rhus tripartitum*) dont la vitalité et le nombre décroissent.

Apparition de *Tamarix* sp., de *Nitraria retusa* et de quelques buissons d'*Atriplex halimus*.

Installation du groupement à *Salsola foetida* et *Zygophyllum gaetulum* à la périphérie de l'aire d'habitat de la savane à *Acacia-Panicum*, dont la végétation est de plus en plus réduite — *Rhus tripartitum* disparaît, *Acacia raddiana* se fait de plus en plus rare.

Disparition totale d'*Acacia raddiana*.

Installation du peuplement de *Limoniastrum ifniense* à l'intérieur duquel se maintiennent quelques touffes de *Nitraria retusa* et des broussailles de *Tamarix* sp., établies sur des buttes de sables mobiles qu'elles parviennent à fixer.

Cette modification progressive de la végétation, qui traduit l'accroissement de la salinité des sols alluvionnaires, a été observée sur une vingtaine de km dans les vallées:

—de l'Oued Erni, à partir d'Hassi el Unguel où apparaissent près du puits les pre-

mières broussailles de *Nitraria retusa*, de *Tamarix* sp. et quelques buissons d'*Atriplex halimus*.

Le groupement se dégrade ensuite rapidement pour faire place à un peuplement, à très faible degré de couverture (moins de 5%), constitué uniquement par 3 espèces:

Suaeda monodiana	3—3
Salsola foetida	1—2
Nucularia perrini	1—1

La salure de l'horizon, fouillé par les racines nourricières (0–0.75) varie de 4 à 7 gr en NaCl par kg de terre sèche.

—de l'Oued Hafra, à partir de Gart el Has où la savane à *Acacia-Panicum*, enrichie d'importantes colonies de *Ricinus communis* et de peuplements arborescents de *Calotropis procera*, commence à régresser: la savane à *Acacia-Panicum* s'appauvrit rapidement. Elle ne tarde pas à faire place, dans la région d'Hassi Sargued Quessad à un groupement végétal, à degré de couverture voisin de 50%, extrêmement simple, constitué par:

Limoniastrum ifniense	4—4
Nitraria retusa	+—1
Tamarix sp.	+
Atriplex halimus	+
Salsola foetida	+
Cistanche phelipaea	+
Cynomorium coccineum	+

La salure de l'horizon superficiel (0–0.75) du sol est élevée. Elle varie de 12 à 25 gr en NaCl par kg de terre sèche.

—de l'Oued Tabha, au Nord de Gara Djebilet ou la savane est remplacée par deux espèces formant un peuplement très dispersé (degré de couverture inférieur à 3%):

Sueda monodiana
Zygophyllum waterlotii

Lorsque la salinité augmente encore, un peuplement de Zygophyllacées, occupe le fond des vallées et le degré de recouvrement est généralement élevé (60 à 75%). Les buissons sont presque toujours établis sur des petites buttes.

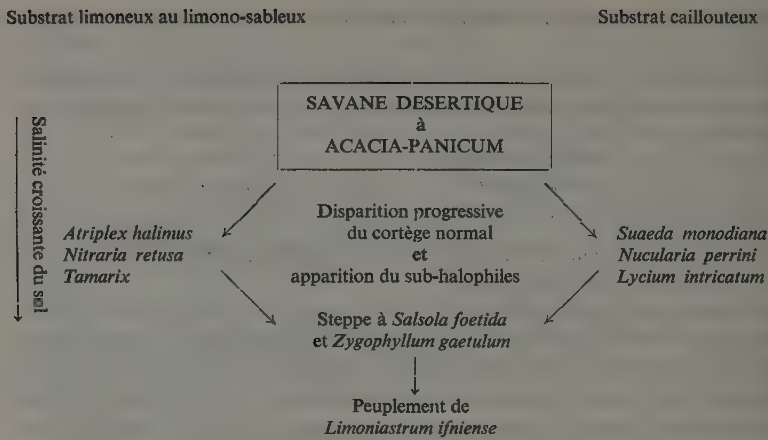
Ainsi *Zygophyllum gaetulum* peuple les vallées:

—de l'Oued Tindouf, au Sud du poste;

—de l'Oued Aouel-ouel, au Sud-Ouest de Tindouf, dans la région de Sebkha Abdallah.

Dans ces deux régions, la salure de l'horizon occupé par les racines nourricières de la Zygophyllacée est très élevée. Elle varie de 17 à 26 gr en NaCl par kg de terre sèche.

Nous avons schématisé ces diverses constatations dans le schéma ci-dessous (Schéma 1).



SCHEMA 1

Evolution de la végétation des lits d'oueds en fonction du salant au Sahara occidental.

En bordure de toutes les vallées, surtout au Sahara espagnol, la steppe à *Arthrophyton scoparium* est installée sur les alluvions argileuses et légèrement salées. Ce groupement végétal est relativement riche en espèces. Toutefois, les graminées vivaces qui abondent dans la savane à *Acacia-Panicum* du lit majeur de l'Oued, font presque toujours défaut.

Rhus tripartitum se maintient çà et là au voisinage des alluvions sablonneuses.

Lycium intricatum devient très fréquent.

Cet aspect de la lande à *Haloxylon scoparium*, spécifique des venues alluvionnaires argileuses, à été observé:

dans la règion d'Atuila, en rive gauche de l'Oued;

dans la région d'Hassi Lassal en rive droite de l'Oued Erni.

Il y a lieu de noter que dans les mêmes localités, *Haloxylon scoparium* forme des peuplements sur les sols les moins chargés en sels (2 à 5 gr en NaCl par kg de terre sèche) et en général à nappe moins profonde.

Lorsque l'alluvionnement et particulièrement l'ensablement sont peu importants, la présence du salant détérmine l'installation d'un groupement halophile caractérisé par les quatre espèces suivantes:

Salsola foetida
Salsola vermiculata
Zygophyllum album
Traganum nudatum

Mais ce groupement, qui peuple en particulier la vallée de la Saoura, a une composition assez instable:

Salsola foetida et *Salsola vermiculata*, accompagnées de *Zygophyllum album* et de *Suaeda mollis* sont dominantes dans la région d'Igli où la salure du sol (0–0.50) varie de 2 à 7 gr en NaCl par kg de terre sèche.

Tamarix pauciovulata apparaît dans le lit majeur de l'oued dès que la salure et l'humidité augmentent.

Le groupement s'enrichit alors de:

Suaeda fruticosa var. *longifolia* dans la région d'Igli.

Suaeda fruticosa var. *brevifolia* (*S. pruinosa*) dans la région de Beni Abbès.

Tamarix articulata, qui peuple toute la vallée de la Saoura jusqu'à Foum el Kheneg, et *Tamarix gallica* ssp. *nilotica* se réfugient sur les tchokolaks en limite des zones alluvionnaires, dans les terrains moins salés mais encore humides.

Atriplex halimus est alors compagne préférentielle. Cette salsolacée se retrouve dans toute la vallée de la Saoura jusqu'àu Foum el Kheneg et même dans quelques stations en bordure de la sebkha el Mellah. Elle indique toujours une diminution appréciable de la salinité des sols.

Zygophyllum album et *Z. geslini* deviennent dominants; par contre, la sècheresse s'accentuant, la salure demeure élevée. Ils existent dans toute la vallée de la Saoura à Igli, Mazzer, Beni Abbès, Kerzaz, Foum el Kheneg, dans le lit sablonneux de l'Oued Messaoud et de l'Oued es Souireg.

Ces diverses constatations ont été figurées sur le Schéma 2.

Steppe à
Arthrophyton scoparium

↓

Groupement à *Salsola foetida*
Salsola vermiculata
Traganum nudatum

Groupement à *Zygophyllum album*
et *Zygophyllum geslini*

→
Augmentation du salant sans élévation du plan d'eau

↓

Groupement à *Tamarix balansae*
et *Suaeda fruticosa*
avec buttes à *Tamarix articulata*
et *Tamarix gallica* ssp. *nilotica*

Augmentation du salant + élévation du plan d'eau ↓

SCHEMA 2

Dans le lit majeur de la Saoura enfin, *Tamarix articulata* occupe une zone restreinte mais peut arriver par place à former une véritable forêt galerie (Tamaricaies d'Abadla). L'aspect de ce groupement lié à des sols à nappe saumâtre peu profonde (2–6m)

varie beaucoup suivant les degrés de saline, Guinet (1954) a pu, en particulier, y distinguer quatre faciès:

le faciès à *Salsola foetida*
le faciès à *Suaeda mollis*
le faciès à *Atriplex halimus*
le faciès à *Suaeda fruticosa*

VEGETATION HALOPHILE DES SEBKHAS

Les Sebkhas sont rares dans les zones envisagées ici et la végétation qui les colonise ne présente pas la même variété que sur les Hauts Plateaux algériens, par exemple, où même les zones les plus salées arrivent à être peuplées par *Halocnemum strobilaceum*.

Dans la sebkha de Tindouf, seule *Salsola tetragona* forme des peuplements assez diffus; il en est de même dans la daya de Bou Garfa au Sud de Tindouf.

La Sebkha de Timimoun est plus remarquable (Guinochet et Quezel 1954). Elle est surtout colonisée par l'association à *Randonia africana* et *Henophyton deserti* qui constitue dans les zones limoneuses faiblement salées, un pâturage fort apprécié des chameaux de l'oasis.

Dans la sebkha elle-même le cortège floristique est constitué essentiellement par:

Randonia africana Coss.
Traganum nudatum Del.
Zygophyllum geslini Coss.
Limoniastrum guyonianum Coss. et D.R.

A la périphérie de la sebkha dans les zones riches en argiles gypseuses s'y ajoutent:

Henophyton deserti Coss. et D.R.
Salsola vermiculata L.
Cistanche violacea (Desf.) Beck

A la faveur de l'ensablement local apparaissent encore:

Cornulaca monacantha Del.
Danthonia forskalii Vahl
Aristida plumosa L.

Dans la portion centrale de la sebkha, dans les zones de la nappe approche de la surface, s'installe l'association à *Tamarix boveana* et *Suaeda vermiculata*. Ce groupement végétal est essentiellement constitué par les espèces suivantes:

1. Caractéristiques de l'Association:

Tamarix boveana
Suaeda vermiculata

2. Caractéristiques des unités supérieures:

Zygophyllum geslini
Limoniastrum guyonianum
Salsola vermiculata
Spergularia salina

3. Compagne:

Cornulaca monacantha
Frankenia glutinosa
Polypogon monspeliensis

VEGETATION HALOPHILE DES REGS ET DES DAYAS

Sur les sols salés de reg, nus ou partiellement ensablés peut s'établir une steppe caractérisée par la présence de Chénopodiacées pérennantes. Ce sont essentiellement:

Salsola foetida
Salsola vermiculata
Traganum nudatum

Sur les terrains les plus secs (solontchaks inertes de la région d'Adrar où la salure est voisine de 5 gr par kg de terre sèche), ce groupement végétal disparaît. Il est remplacé par un peuplement presque pur et extrêmement diffus de:

Zygophyllum album
Zygophyllum geslini

Sur les hamadas siliceuses, un peu salées, la steppe à *Haloxylon scoparium* se développe vigoureusement. Elle occupe tout le Sud-Est du Sahara espagnol jusqu'au massif primaire de Langueirinat où elle se présente avec une vitalité optimale. Dans cette région, la monotonie du paysage végétal n'est interrompue que par les vallées occupées par la savane à *Acacia-Panicum*, qui traversent la hamada d'Ouest en Est. Les manifestations du salant y sont extrêmement faibles.

Ce groupement à *Haloxylon scoparium* a été seulement décrit par Guinet (1954) sous le nom de pseudo-steppe à *Haloxylon scoparium* qui l'a reconnue dans les régions suivantes:

Taghit sur le reg;
Igli;
Ouarourout, sur le reg grossier et sablonneux;
Zeghamra, dans les monts d'Ougarta.

Il est caractérisé par un petit nombre d'espèces et par l'abondance des *Aristida*.

Les dépressions de la hamada où se rassemblent les eaux de ruissellement, qui apportent les alluvions sablonneuses, limoneuses et argileuses, ont des sols généralement plus consistants, qui ne sont plus mobiles en surface.

Ces dépressions ou dayas, dont les dimensions sont extrêmement variables, sont rarement halophiles. Les ceintures de *Bubonium graveolens*, d'*Anvillea radiata* ou d'*Anastatica hierochuntica*, qui les entourent, traduisent une humidité au moins transitoire, favorable aux germinations.

Lorsque le salant se manifeste, les espéces érémitiques halophiles s'établissent. Le groupement végétal est toujours pauvre. Dans la daya, située au km 84 de la piste Adrar–Reggane par exemple, la végétation est constituée uniquement par 4 espèces:

Salsola vermiculata var. *villosa*
Fagonia oliveri var. *jolyi*
Brocchia cinerea
Morettia canescens

Seule *Salsola vermiculata* indiquant d'ailleurs la présence du salant.

CONCLUSION

La flore halophile du Sahara occidental est donc très pauvre. Elle est constituée par une trentaine d'espèces spécialisées représentant environ le dixième des espèces reconnues soit par Guinea (330 en 1952 dans le Sahara espagnol) soit par Guinet et Sauvage (250 en 1953 dans les hamada sud-marocaines).

Cependant des groupement végétaux, ont pu être déjà individualisées. Leur nombre est limité en raison du rôle prépondérant du facteur édapho-climatique. Mais l'insuffisance des prospections phyto-sociologiques effectuées jusqu'à ce jour dans ces régions éloignées, d'accès souvent difficile, ne nous permettent encore que d'avancer un tableau provisoire et incomplet de ce type de végétation.

Ici, encore, seules des études plus approfondies seront susceptibles d'établir définitivement un inventaire significatif des groupements halophiles du Sahara occidental.

OUVRAGES CITES

Guinea, E., 1949, El Sahara espanôl, *Consejo superior de investigaciones scientificas, Instituto de Estudios africanos*, Madrid, 808 pp.

Guinet, Ph., 1954, *Carte de la végétation de l'Algérie*, Feuille de Beni-Abbès.

Guinet, Ph. et Sauvage, Ch., 1954, Les Hamada Sud marocaines. Botanique,*Trav. Inst. Sc. Chérifien*, série générale, no. 2, pp. 75–167, 12 pl., 1 carte.

Guinochet, M. et Quezel, P., 1954, Reconnaissance phytosociologique autour du Grand Erg occidental, *Trav. Inst. Rech. sahariennes*, **12**, 11–27.

A SENSITIVE EVAPO-TRANSPIRATION GAUGE

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ABSTRACT

Equipment is described for the separate assessment of (a) transpiration per unit area of crop and (b) evaporation from the soil surface, from crops grown at commercial spacings and under normal methods of management. The equipment is based on the application of measured amounts of water to each unit area of soil through slow-running nozzles; assessment of gain or loss of water from the rooting zone, by measuring soil moisture tension; and the measurement of drainage water, which is caught in shallow trays buried deeply in the soil below the rooting zone. The equipment allows free run of roots, thus avoiding the edge effects of normal enclosed lysimeters.

With this gauge it is possible to calculate the daily evapo-transpiration from unit areas of land which are maintained virtually at field capacity, and also to separate soil evaporation from transpiration at various degrees of plant cover. These figures can then be compared with measurements of various climatic factors to check correlation between actual and calculated evapo-transpiration rates. The data can also be used for the accurate control of a series of different water-regimes to ascertain the moisture deficit at which growth and yield are affected by lack of water, thus specifying the stage in the drying cycle at which irrigation water should be applied, at each stage in the growth of a crop, to prevent yields from being depressed by drought.

Results for tomatoes grown in a heated glasshouse in two gauges, each 210 × 180 cm in area, surrounded by several guard rows on three sides and exposed to a concrete path on the fourth, showed (a) a very high correlation between daily water use per plant and evaporation as measured by evaporimeters, (b) differences of nearly four times in water use between one day and another, depending on the weather, (c) transpiration at the rate of nearly 9 mm water per day in hot sunny weather, and (d) water consumption by plants in the guard rows at a rate more than double that of plants within the cropping area.

As Oppenheimer has pointed out (1953), the key requirements for efficient use of water in irrigation are a knowledge of (a) when to apply the water and (b) how much to apply. The answer to the first question can only be obtained by subjecting plants to a series of carefully-controlled water-regimes, which will show the latest stage at which irrigation must be applied if growth and yield are not to be reduced by lack of water. The second question, 'how much water to apply', requires an assessment to be made of the amount of water which the rooting zone can absorb before any runs through to drainage. For this it is convenient to use the concept of the water deficit, i.e. the amount needed to restore soil moisture to field capacity. This can be

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estimated directly, but laboriously, by the analysis of numerous soil samples; or, less accurately, by *in situ* measurement of moisture conditions (e.g. moisture tension) at a number of check points; or, indirectly, by estimating the rate at which water has been lost from the soil surface (by evaporation) and from the plants (by transpiration) since the soil was last at some known moisture condition, such as field capacity. Since evaporation from soil and plants are both affected by meteorological conditions, a logical approach is to seek some correlation between measurable factors of the plant environment and the rate of water loss, so that the amount of evapo-transpiration can be estimated from these measurements, modified where necessary by factor (s) based on characteristics of the species, stage of growth, depth of rooting, and nature of the soil. Considerable success has been achieved, notably by Penman (1953), in relating meteorological conditions to the rate of evaporation from a free water surface and the evapo-transpiration from a full cover of plants growing with free access to readily available supplies of water. However, Oppenheimer's work on the water relations of plants grown in semiarid conditions has shown that there is also a need for a sensitive method of assessing the rate at which various plants use water, as the soil dries out and water becomes less readily available to the roots, since under these conditions potential and actual evapo-transpiration rates diverge.

In view of Professor Oppenheimer's interest in this subject it is a pleasure to have the opportunity of describing equipment that has been developed to measure the rate at which water is lost per unit area from plants set out at normal spacings, and grown under conditions similar to those of field production of the crop concerned. The technique enables separate assessments to be made of transpiration from the plants and evaporation from the soil surface, at successive stages in the life of the crop, when the soil is shaded by plants to varying degrees.

The most perfect instrument for measuring evapo-transpiration from a growing crop is probably the large weighable lysimeter developed by the National Institute of Agricultural Engineering, to hold 2600 kg of soil and weighable to an accuracy of 5 grammes (Morris 1959), but such instruments are too costly to be used in large numbers and may require some engineering facilities for maintenance. Small weighable lysimeters, though useful, suffer from the disadvantage of having a relatively restricted volume of soil with a large edge effect, while it is difficult to expose the plants to exactly the same evaporating conditions as those experienced on a field scale.

As a cropping season advances, changes can occur in the soil/water system as follows:

Gains — Additions of water from above, by irrigation or rainfall; or from subterranean sources such as a rising water table (which are disregarded in this paper).

Losses — Water leaching down beyond the rooting zone to drainage; evaporation from the soil surface; transpiration from the plants; and water conserved in the plant tissues (i.e. the bulk of the increase in fresh weight).

Gain or loss — Increases or decreases in the reserve of available soil water from day to day, or as the season advances.

Work in this Department on the water relations of plants grown under glass has led to the development of a gauge in which it is possible to measure each of these components to within an accuracy of 1-2 per cent and thus arrive at a close daily estimate of the total evapo-transpiration from a unit area of plants grown at normal spacing. It seems possible that the gauge could be used for the same purpose in the field, especially where rainfall is infrequent.

CONSTRUCTION

A basic feature of the evapo-transpiration gauge is the very slow application of measured amounts of water or diluted nutrient each day, at a series of points spaced over the experimental area, using a type of nozzle with an output of about 1.1 litres per hour at a head of about 150 cm. When a trickle of water is applied to the soil from a small nozzle a cone of wetting develops below the point of application, the shape of the cone being characteristic of the soil type (Hudson, in press). The surface of the gauge is covered by a series of trickle nozzles spaced rather closer than the widths of their cones of wetting, so that the bases of neighbouring cones overlap slightly (Figures 1a and 1b). The characteristics of cones of wetting in any particular soil can be ascertained by allowing a few nozzles to drip to excess on a dry soil and excavating trenches across the cones, the edges of which are clearly demarcated. In the coarse sandy loam soil at Sutton Bonington the cones of wetting develop to a width of about 90 cm at a depth of 75 cm and trickle nozzles in the evapo-transpiration gauge are spaced for convenience at about 60 × 50 cm apart.

A number of shallow lysimeter trays are buried 75 cm deep in the soil, at the depth to which the rooting zone normally extends, arranged so that one tray underlies each cone of wetting (Figures 1 and 2). Each tray is about 59 × 52 cm in plan and 15 cm deep, with a drainage hole in the centre. The joints between trays are covered with metal caps to prevent drainage water from running to waste down the joints, while an apron of polythene sheet directs water downwards into the outer trays in each series (Figure 3). The drain pipe from each tray discharges separately into a deep trench, where the effluent can be measured. Although there is a slight overlap between neighbouring cones, all drainage water from any particular nozzle runs down the centre of its cone of wetting and is collected by the tray under that nozzle.*

Two gauges are at present in use, each containing twelve plants served by twelve nozzles and underlaid by twelve trays (i.e. a surface area of about 210 × 180 cm) but others are being constructed. Water or diluted liquid fertiliser is supplied separately to each nozzle from a calibrated container, to which it is connected by rubber tube.

* The trickle irrigation nozzles and equipment for supplying variable dilutions of liquid fertilisers were purchased from Messrs. Cameron Irrigation Co. Ltd., 208 West End Lane, London, N.W. 6. Soil Moisture Tensiometers were purchased from Messrs. A. Gallenkamp & Co. Ltd., Sun Street, London, E. C. 2.

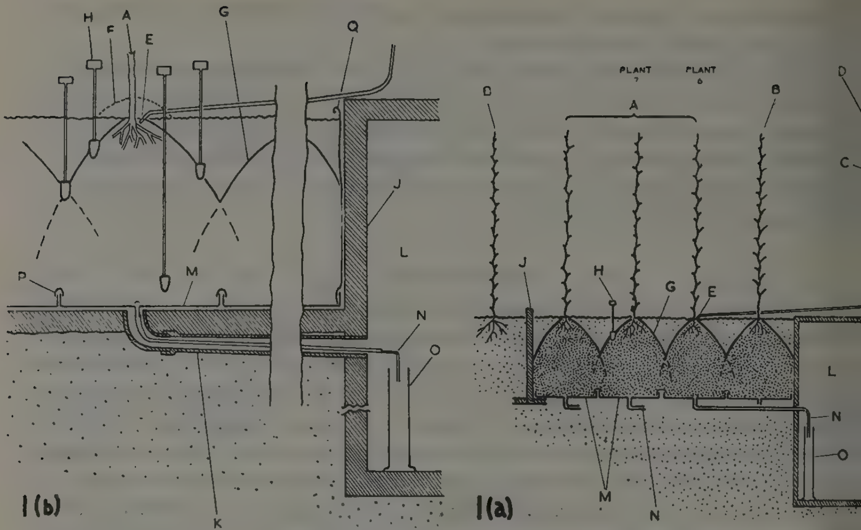

Figure 1

Diagrams illustrating the construction of the evapo-transpiration gauges showing three experimental tomato plants (A), and two guard plants (B); a graduated feed bottle (C) filled from a water supply line with tap (D) and serving a trickle nozzle (E) buried near the surface and covered with a heap of coarse grit (F) to reduce surface evaporation; cones of wetting (G) develop under each nozzle, the moisture condition of each cone being assessed by soil moisture tensiometers (H) embedded at various places in and around the cones; each gauge, holding twelve plants, is enclosed in a concreted tank, with retaining walls (J) to separate treatments, and a base perforated by twelve drain pipes (K) leading into a deep observation trench (L). Twelve shallow metal lysimeter trays (M) are in position at the base of each tank, with a plastic outlet pipe (N) from each tray threaded through the drain pipe to a container (O) in the trench for collecting effluent. Joints between the trays are covered with metal caps (P) and each tank is lined with a polythene apron (Q) to lead water into the outer tray. (N.B. The use of plastic pipes threaded through permanent larger bore drain pipes facilitates inspection of joints or removal of faulty trays).

(a) Simplified section through one of the gauges.
(b) Details of part of a gauge.

The nozzles are buried just below the surface of the soil and covered by a few handfuls of coarse grit, to reduce evaporation from the small wet patch around the nozzle (Figure 4).

The most difficult component of the soil water system to measure accurately is the net daily gain or loss of water from the rooting zone, especially in a system in which the geometry of water distribution is complicated by the presence of cones of wetting separated by areas of soil containing little or no available water. The system adopted is to embed five soil moisture tensiometers in and around selected cones of wetting, so that the porous pots of three of the tensiometers are in the fringes of the cones.

Figure 2
One of the gauges excavated to show the surrounding brick wall and nine of the twelve lysimeter trays in position, below the rooting zone. Note the central drain from each tray and the shallow layer of broken crocks to provide uniform drainage in place in some of the trays.

Figure 3
One of the plots being refilled with soil after insertion of an apron of polythene to lead drainage water into the outer trays.

Figure 4

View of the surface of one of the gauges showing the bases of tomato plants, the rubber tubes leading to individual trickle nozzles, the vacuum gauges of soil moisture tensiometers, and heaps of coarse grit used to cover the sites of nozzles and reduce surface evaporation. The soil between nozzles is dust dry.

From a knowledge of the characteristic curve relating soil moisture tension to percentage of soil water (Majmudar and Hudson 1957), it is possible to calculate the losses or gains of water represented by any particular changes in tension, although these changes are of course small from day to day where water is applied daily to replenish the cones of wetting to field capacity.

Between the nozzles, the soil surface becomes completely dry and friable to a depth of many centimetres, but small wet patches tend to develop around the site of each nozzle. To reduce evaporation from these places each site is covered with a few handfuls of coarse grit. The whole area of the gauges is surrounded by several guard rows at normal spacings except on the side next to the observation trench, the concrete top of which is used as a pathway (Figure 5).

The equipment thus enables the following items to be measured to a high degree of accuracy over a series of adjacent areas, each about 300 sq. cm and each containing one tomato plant:

Water applied to the rooting zone
Water lost to drainage
Net increase or decrease in reserve of soil water

Figure 5

Tomato plants growing in one of the gauges (left) with the concrete cover of the observation trench (centre) and rack holding graduated feed bottles (right).

OPERATION OF THE GAUGE

Up to the present these gauges have been used to measure actual transpiration from plants grown with free access to readily-available water, i.e. in soil maintained virtually at field capacity by applying water daily in slight excess of expected requirements. Separate tests had shown that excess water applied on any particular day caused rapid drainage during the next 24 hours but little thereafter. Thus the difference was taken between water applied one day and the water caught in the drain by the following morning.

Since the whole of the soil surface was dust dry, it was assumed that evaporation from the soil was negligible, and the differences between the above items were taken as the amount of water transpired by the plant, plus water incorporated into the plant tissues, calculated for periods of one day. Assessments of increases in fresh and dry weight, based on non-destructive measurements, enable the latter

component to be estimated (Salter 1954-8), but the amount is of a negligible order when considering water use on a day-to-day basis.

To assess the rate of evapo-transpiration when the surface of the soil is wet instead of dry (e.g. as in surface irrigation), all that is necessary is for a number of sites to be moistened daily by applying a measured amount of water through a fine spray in addition to the main watering through the nozzles. The difference between total evapo-transpiration in the two sets of systems, one with a dry soil surface and the other with a wet surface, gives evaporation from the soil surface, which can then be used to show the effect of increasing crop coverage on water loss.

In using this gauge to measure daily consumption of water the essence of the method is to apply a little more water each day than the plant will transpire, to give a measurable amount of drainage. This can be done by trial and error, applying water daily until the drain from each tray starts to run, but the method leads to over-wetting of the centre parts of the cones of wetting, and poor growth of roots, in all but very light soils. It is more satisfactory to use some system of estimating the daily requirement and applying a slight excess, making constant adjustment as experience dictates until some useful correlation with measurable climatic conditions emerges to serve as a more precise guide. The equipment will itself show at once if too much water is being applied (by excessive drainage), or too little (by a rise in the tensiometer readings). Earlier work at Sutton Bonington, using a much less sensitive method of measuring transpiration, had indicated a close correlation between water use and evaporation as measured by a type of evaporimeter developed by Stanhill (1958). This correlation has been amply confirmed by results from the more sensitive evapo-transpiration gauge (Figure 6).

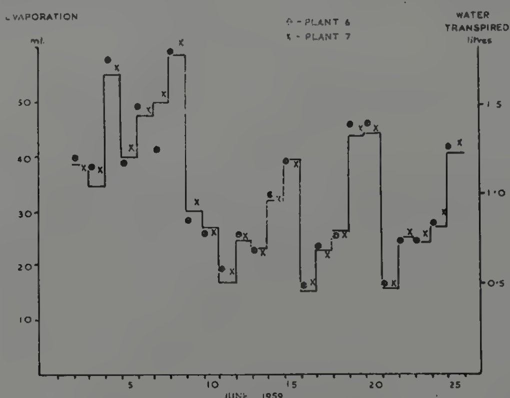

Figure 6

Correlations between daily water use of neighbouring tomato plants and daily measurement of evaporation. It is interesting to note the differences between dull cool days (e.g. 11, 16 and 21 July) and bright sunny ones (e.g. 4 and 8 July).

Histograms — Average of two Stanhill evaporimeters suspended at 15 cm above the plants

• — Water use of plant 6 in one gauge (Figure 1a)

x — Water use of plant 7 in same gauge (Figure 1a)

EXPERIMENTAL RESULTS

It may be of interest to quote some of the results of work in 1959.

1. Since deeply-buried lysimeter trays are used to avoid the complication of edge effects, the assumption is made that there may be some exchange of roots between one cone of wetting and another, but that such exchange will be approximately equalised between neighbouring plants. This appears to have been justified since the difference between water consumption of neighbouring plants was small (Figure 6).

2. Plants in the guard rows, next to the concrete path, used more than twice as much water as the experimental plants. This emphasises the importance of adequate guard rows in work on water relations, since the rate of water use in small lysimeters may bear little relation to consumption of crops at normal spacings unless the lysimeters are surrounded by sufficient guard plants of the same stature.

3. During a period when weather was very variable, transpiration on hot, sunny days was nearly four times as great as on cool, dull days (Figure 6).

4. An obvious use of this type of equipment is to seek correlations between daily water use and various measurable factors of the plant microclimate. In the present series, useful correlations have been found with incoming energy (as measured by a Gunn Bellani radiation indicator) and total light (as measured by a Megatron photometer) but the outstanding correlation was with evaporation as measured by the average of two Stanhill evaporimeters suspended about 15 cm above the growing plants.

DISCUSSION

With this equipment it is possible to measure the rate of water loss, from the soil and from the plants separately, at successive stages in the life of the crop, and calculate the degree of correlation between either, or both, of these components of actual evapo-transpiration with any measurable meteorological conditions. By irrigating each day, and thus maintaining a series of gauges virtually at field capacity, it is possible to measure consumptive use of water on a daily basis and thus establish a running total of potential evapo-transpiration.

It happened that commercial spacing of tomato plants was about the same as the size of the cones of wetting and it was therefore convenient to grow one tomato plant at each nozzle. However, other experiments have shown that tomatoes will grow equally well at other nozzle spacings, even where plants were up to 30 cm from the nearest nozzle. In such cases, asymmetrical root systems develop but with no apparent effect on growth. Thus it appears that this system of spot irrigation could be used for other crops that require quite different spacings. It could, for instance, no doubt be used for the long-term study of the water relations of fruit trees provided a sufficient area was initially equipped with the buried lysimeter trays, and thus help to answer some of the questions propounded by Oppenheimer in connection with citrus trees (1953).

The gauge can also be used to find out when the amount of leaf area is equivalent to a 'uniform cover of green vegetation', after which further increases of leaf would not be expected to increase water use relative to a free water surface, but the data for tomatoes in the present experiments are not yet available.

To ascertain the critical condition at which plants are affected by shortage of water all that is necessary is to apply water to various plots at a series of pre-determined deficits, as shown by the cumulative water losses from plants in the gauge with continuous access to freely available water, preferably in a series of plots separated by suitable barriers to prevent roots from straying into soil where moisture conditions are different. In that way information can be obtained on the timing of irrigation and the amount of water needed at each irrigation, either to obtain maximum yields where water is not limited (as in our own work with high value crops grown under glass), or to produce the most economic yield where the cost or availability of water is a limiting factor.

ACKNOWLEDGEMENT

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REFERENCES

Hudson, J. P., 1958, (in press), Characteristics of the trickle irrigation system, *Rep. XVth Int. hort. Congr., Nice.*

Majmudar, A. M. and Hudson, J. P., 1957, The effects of different water regimes on the growth of plants under glass, II, *J. hort. Sci.*, **32**, 201-213.

Morris, L. G., 1959, A recording weighing machine for the measurement of evapotranspiration and dew fall, *J. agric. Engng. Res.*, **4**, 161-173.

Oppenheimer, H. R., 1953, Water requirement and irrigation of citrus groves, *Rep. XIIIth Int. hort. Congr., London*, 1952, **2**, 1267-1274.

Penman, H. L., 1953, The physical bases of irrigation control, *Rep. XIIIth Int. hort. Congr., London* 1952, **2**, 913-924.

Salter, P. J., 1954-1958, The effects of different water-regimes on the growth of plants under glass, I, III & IV, *J. hort. Sci.*, **29**, 258-268; **32**, 214-226; **33**, 1-12.

Stanhill, G., 1958, An irrigation gauge for commercial use in field and glasshouse practice, *J. agric. Engng. Res.*, **3**, 292-298.

NEWER METHODS IN CYTOLOGY

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ABSTRACT

1. Studies in modern cytology are based on precisely localised chemical staining reactions or biophysical investigations of molecular or micellar orientation.
2. For such studies it is essential to produce no artifact, such as coagulation or diffusion.
3. A new method is described which seems to fulfil these requirements.
4. The application of this new procedure to studies by the mewer methods of autoradiography, polarization and fluorescence microscopy is discussed.

INTRODUCTION

The need for improved preparatory procedures

By about the end of the second decade of the present century it had become very difficult to make appreciable advances in the general study of the plant cell. This was due to the fact that cytologists were being faced with problems which required for their elucidation such chemical and physical techniques as were not then available. Indeed it may not be unfair to say that the cytologist had progressed further than the biochemist and biophysicist and had to wait for them. Thus the period between about 1930 and 1940 saw cytologists concentrating on the study of cytogenetics, namely the correlation of descriptions of chromosome morphology and behaviour with Mendelian genetics (see Darlington 1937). It is only in the last ten years that the requisite biophysical and biochemical methods have become available; these were adopted eagerly by students of animal cells but not so actively by plant cytologists. This was because the newer techniques required very precise, almost ideal, methods for preparing the tissues; for animal cells the freezing and drying method was available (see Bell 1952) but it did not give satisfactory results with plant cells (see Jensen 1954a, Chayen 1952).

For the newer cytological methods life-like preservation is essential. Thus, for example, it is useless to have a histochemical method for localising a particular sub-

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stance or enzyme precisely, to $\pm 0.1\mu$, if the preparatory procedure has permitted it first to diffuse from the cytoplasm to the nucleus (Chayen and Jackson 1957, Chayen, in press) or even right out of the cell into the intercellular spaces. Thus Mancini (1948) has claimed that glycogen diffuses out of liver cells after chemical fixation. Moreover, examination of the birefringence of structures which, in life, contain anisotropic fibrils is unlikely to yield very useful results if the molecules have been disorganised during fixation or dehydration. Hence as the methods of investigation become more precise so the errors due to artifacts produced during the preparation become more serious. However, it may not be possible, merely by inspection, to decide how much damage has been done by the processing so that it is becoming increasingly advisable to require as criterion of a satisfactory preparation, that it should show life-like preservation of the obvious structures of the cells (see Chayen and Jackson 1957). It may then be reasonable to argue as follows: if the cellular structure is as in life then it is possible that the chemistry of the cell is also preserved; but if the structure is not life-like then the probability must be that the chemistry too is distorted.

The freezing and drying method for animal cells

In the freezing and drying technique, used so successfully for animal cells, the tissue is "quenched" at about $-190°C$ so that the intracellular water sets, almost instantaneously to a microcrystalline "glass". The water is then "distilled" off from the ice at about $-40°C$ under vacuum; the fully dehydrated tissue is embedded in paraffin wax and sectioned in the usual way. The sections are treated with xylene to remove the wax and they are then immersed in absolute alcohol to fix the protoplasm. It might be thought that this treatment with fat solvents will remove only free fats, such as triglycerides, but it has been shown (Chayen and Miles 1953) that plant meristematic cells contain granules which resemble mitochondria and which are destroyed by fat solvents. The importance of lipids as structural components of cellular organelles is becoming recognised only slowly but this is an important reason for trying to avoid the use of fat solvents at any stage of the preparatory procedure.

The primary purpose of the present communication is to describe a new method for preparing plant tissues for examination by the newer histochemical and biophysical methods. It is hoped to demontrate that this technique has the advantages of tissue freeze-drying with the additional virtues of providing life-like sections of plant cells and of avoiding the use of fat solvents.

The new method

This procedure differs from the freezing and drying technique most strikingly in the rate at which the tissues are frozen. The plant material can be used either fresh or pretreated for about 2 hours with a 5 per cent aqueous solution of polyvinyl

alcohol (PVA; Bush, Beach and Gent, Ltd., London, type M 05/140), an inert stabiliser of cellular detail (Chayen and Miles 1953, 1954). The tissue is chilled by pressing against the side of an empty tube at between $-40°$ and $-70°C$. In its simplest form the method is as follows: the tubes (3" by 1" specimen tubes) are cooled overnight by packing them, corked, in solid carbon dioxide in a Dewar flask; they are removed, uncorked, the tissue is pressed gently but firmly against the side or bottom of the tube, which is recorked and replaced as speedily as possible in the Dewar flask. A variant of this is to stand the tube in a bath containing a freezing mixture at a known and constant temperature.

The justification for this relatively slow chilling, apart from the results, is two-fold. Firstly, when tissues have to be frozen for storage, alive, it is done at this sort of rate, not by quenching. Examples of this are the methods for preserving tumor tissue and skin for subsequent transplantation (Billingham 1954, Smith 1954) which are almost identical with this chilling procedure, and the rate of freezing used for preparing viable sperm and bacteria (see Smith 1954). The second justification stems from the work of Asahina (1956) who studied naturally isolated plant cells which were frozen and thawed on the stage of his microscope. He found that if the cells were frozen under optimal conditions the extracellular water froze first and desiccated the supercooled intracellular protoplasm. He then induced ice crystals inthe intercellular spaces but the cytoplasm either never froze, remaining supercooled even at considerably lower temperatures than were expected, or it froze with too low a concentration of water to cause any damage. Such cells recovered completely on being thawed (also see similar work in Smith (1954)).

After the tissue has been chilled it can be stored at —40°C or preferably packed round with solid carbon dioxide. When it is to be sectioned it is removed from the tube and placed, with minimum delay, on to a chilled block-holder of the standard type. This can be effected most easily by standing the metal holder in a vessel containing a constant freezing mixture of solid carbon dioxide and alcohol or acetone (about -60 to $-70°C$). On the top of the holder is made a small flat mound of ice; another drop of water is added and, as it freezes the frozen tissue is placed on it and thus becomes cemented on to the block-holder. Much care is needed to ensure that the tissue does not thaw, or warm up appreciably during all this handling.

Sections are cut at about 8μ on a normal Cambridge "rocking" microtome in a refrigerated cabinet maintained at between about -20 to $-22°C$ (see Coons *et al.* 1951). The knife is kept at a lower temperature than is the cabinet by attaching to it a brass basket containing solid carbon dioxide. A glass "guide", to stop the sections from curling, should be used. This consists of a small slide, or coverslip, which can be brought to lie flat against the knife with only a narrow gap between them, down which the section moves as it is cut off the tissue. A suitable size for the gap is the thickness of a strip of Selotape.

The sections, mounted dry on a slide, can be dried in a refrigerator or they can be placed over phosphorus pentoxide in a vacuum desiccator which is stood in a bath containing solid carbon dioxide and alcohol. It is evacuated and the sections are left to dry (e.g. some hours or overnight), after which they are allowed to warm up to room temperature before the vacuum is released. They may then be stored in the laboratory, preferably in some sort of desiccator.

RESULTS

In demonstrating a new technique it is necessary first to show that it yields results at least comparable with the best obtainable by well established methods. It may then be explained in which ways the new procedure is an improvement on the old.

Studies in general histology

Sections have been cut of roots of *Vicia faba* and of *Allium cepa;* the stem of *Ranunculus repens* and of *V. faba:* and of the leaves of *R. repens*, *Narcissus poetae*, *Sorghum*, *Dryopteris filix mas* and *Ceterach officinarum*. In no case were any signs of shrinkage or distortion seen. Thus in the roots, cell lineages were apparent and, for example in onion roots, the meristematic primordia were obvious (Figure 1). In the leaves, guard cells and air spaces were well preserved (Figure 2) and in all, roots stems and leaves, the results have been as good as, or rather better than, by any established procedure.

Cytological studies

Investigation with polarised light. The birefringence of all cell walls is retained by this technique. Thus although the secondarily thickened walls are brilliantly birefringent, strong birefringence is found even in the walls of more parenchymatous cells both in roots and leaves (Figures 3 and 4). Anisotropy of what seems to be the simple primary cell wall has also been observed.

In leaves the weak birefringence of chloroplasts has been detected while in certain exceptional cases (see below) birefringence in dried protoplasm has been demonstrated. In a study with Professor Oppenheimer (in preparation) birefringent globules or "vacuoles" were found in the cells of certain leaves; these structures were not preserved by fixation and embedding in paraffin wax.

Cytoplasmic structures. The most important improvement produced by this technique, however, is the preservation of the cytoplasmic granules of meristematic cells. It has been clear for many years (Guillermond 1941) that morphogenesis must be associated with changes in the cytoplasmic organelles. Such changes are well known in animal tissues but should be even more obvious in plants which show alteration from proplastid to leucoplast or chloroplast as well as variation in the chondriome and in vacuolar organization. Yet there has been no technique avail-

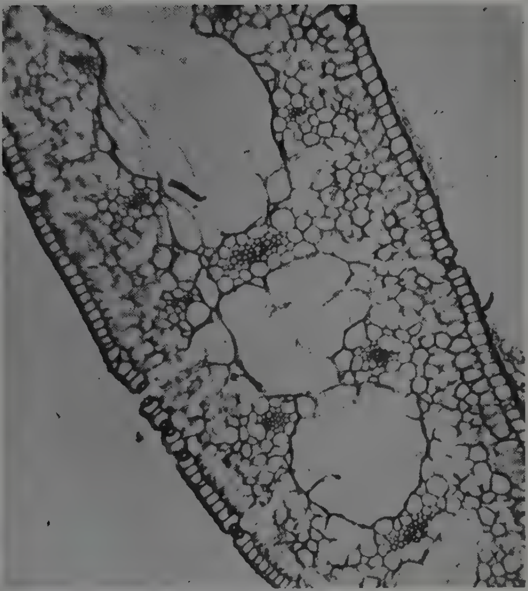

Figure 2

Transverse section through leaf of *Narcissus*. Visible light; × 65. To show undamaged air-spaces.

Figure 1

Composite photograph of a longitudinal section through a root of *A. cepa*. Dark ground illumination; × 100. To show the preservation of morphological detail and cell lineages.

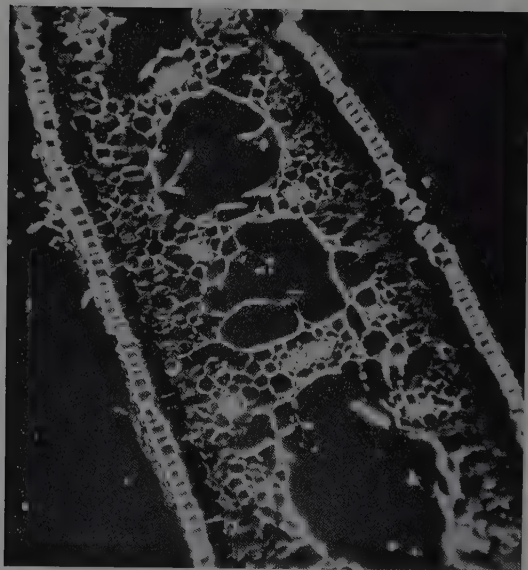

Figure 3
As Figure 2. Crossed polaroids.

Figure 4
Part of a longitudinal section through a root of *A. cepa*. Crossed polaroids; × 600. To demonstrate molecular orientation in walls of relatively meristematic cells.

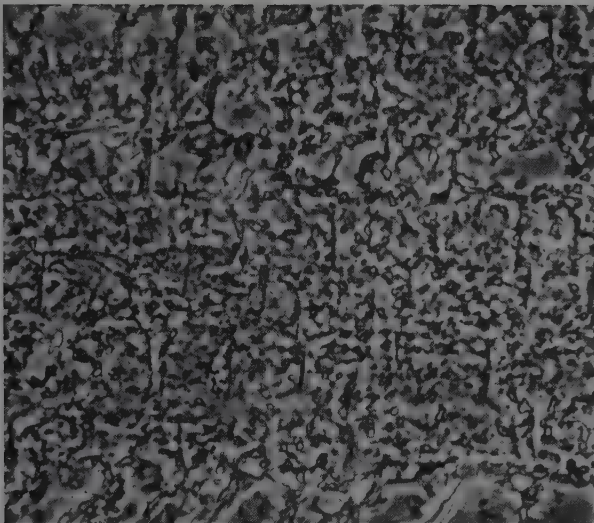

Figure 5
Cells in a longitudinal section of an onion root. Photographed with phase contrast illumination; × 600. To show the cytoplasmic "granules" and mitochondrial structures.

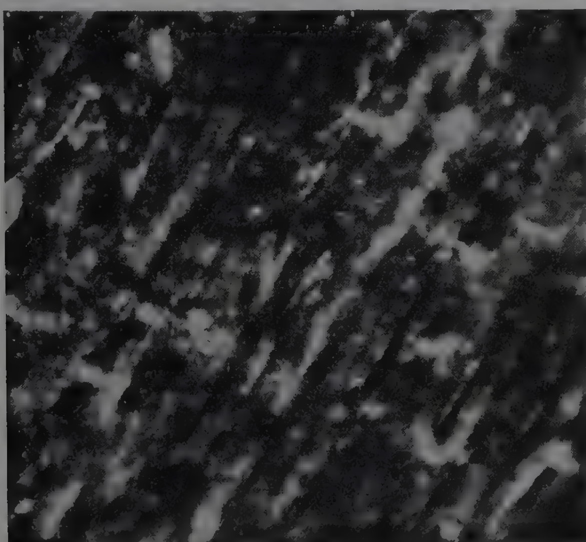

Figure 6
Cells in a longitudinal section of an onion root. Photographed with dark-ground illumination; × 635. To show small white "specks" which may be microsomes.

able by which such changes could be studied since none preserved these delicate structures. Attempts to do this over a number of years have produced unreliable procedures which only occasionally, if ever, gave a true picture of the structure of plant cells (e.g. see Zirkle 1928, Newcomer 1941, Chayen 1949, 1952b, Chayen and Miles 1953, 1954, Chayen and Gahan 1959). However, this new technique, if performed carefully, seems to yield sections in which all the cytoplasmic organization is preserved (Figure 5), giving a picture of the plant cell which agrees well with that obtained from living cells (e.g. see Chayen 1952a; in press).

The occurrence of "microsomes". Before the advent of electron microscopy, cytologists differentiated between large particles, such as mitochondria, and those bodies which could not be resolved by the light microscope but whose presence could be detected by dark-ground illumination. Such a system of microscopy, by virtue of a form of the Tyndall effect, permitted the demonstration of structures which were less than 0.1μ in diameter; such bodies were called "microsomes" (Baker 1946). At the present time this term is used, generally, for small discs some 0.02μ in diameter (Hackett 1955), and few modern cytologists have seen these structures, whatever they be called, which can just be visualised by dark-ground illumination. This was because fixation, with its attendant coagulation of the cytoplasm, confused the picture obtained by this type of microscopy. The new technique, however, produces no coagulation so that such organelles can be seen when viewed by means of a dark-field illuminator (Figure 6). Indeed investigations of this sort ultimately may allow the cytologist to check, by light microscopy, at least some of the results obtained by electron microscopy.

Identification of freely-soluble substances

An investigation has been made by Gahan (unpublished data) of the translocation of material from leaves and along the stem. This has been done in two ways: in some experiments rhodamine B has been introduced into the leaf and stem and its distribution has been studied in sections by the use of fluorescence microscopy (Figure 7). In other experiments leaves of *N. poetae* were grown in the presence of $C^{14}O_2$. When such leaves were cut, they "bled" profusely, losing most of their radioactivity, but if they were cut under PVA and were treated with it, they retained their radioactive matter. Moreover, water soluble material, preserved by this preparatory technique, was kept in the cells during autoradiography by the use of apposition autoradiography (Figure 8). In this method the section is mounted on one slide and the stripping film is placed on a coverslip held, for convenience, on a second slide. The two slides are hinged by means of strong adhesive tape and pressed closely together to expose the photographic film to the radiation emanating from the section. After a suitable time the coverslip, still held on the second slide, is developed and fixed, with the section still hinged to it but not immersed in the solutions.

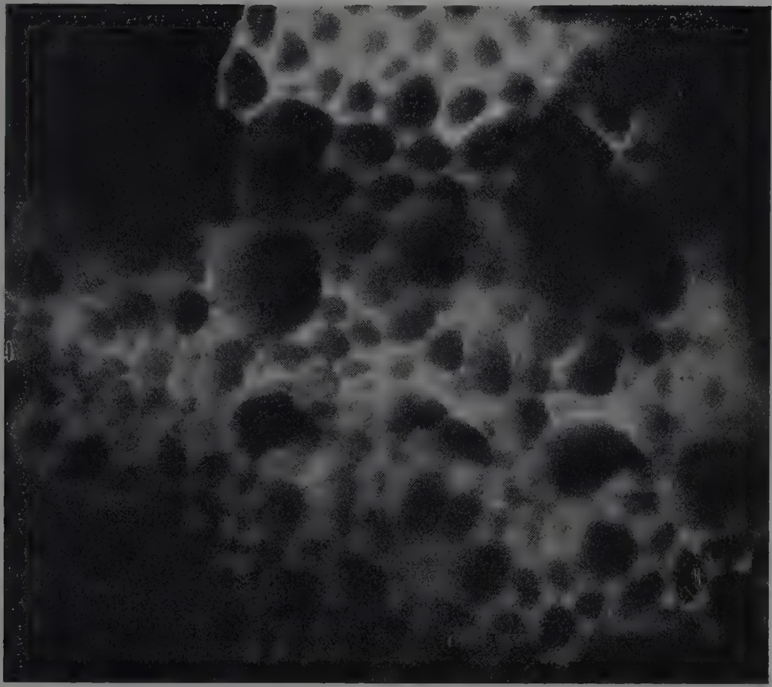

Figure 7

Fluorescence photograph to show localization of a fluorochrome, rhodamine B, in a transverse section through a leaf of *Vicia faba*. × 255.

The coverslip plus film is then recombined with the section; this process requires careful orientation (Gahan, unpublished data).

To demonstrate sites of water-soluble, labelled substances, another section is treated with water before preparing the autoradiograph, which can be made in the normal way.

Studies of dried tissues

In recent investigations performed in collaboration with Professor Oppenheimer, it was important to be able to study sections of plant leaves which had been kept at various relative humidities. Aqueous fixation, therefore, could not be used and in practice alcoholic fixatives followed by embedding in paraffin wax also destroyed the very striking differences found in these cells when kept at these various humidities. The new method involving initial freezing of the tissue and then sectioning and

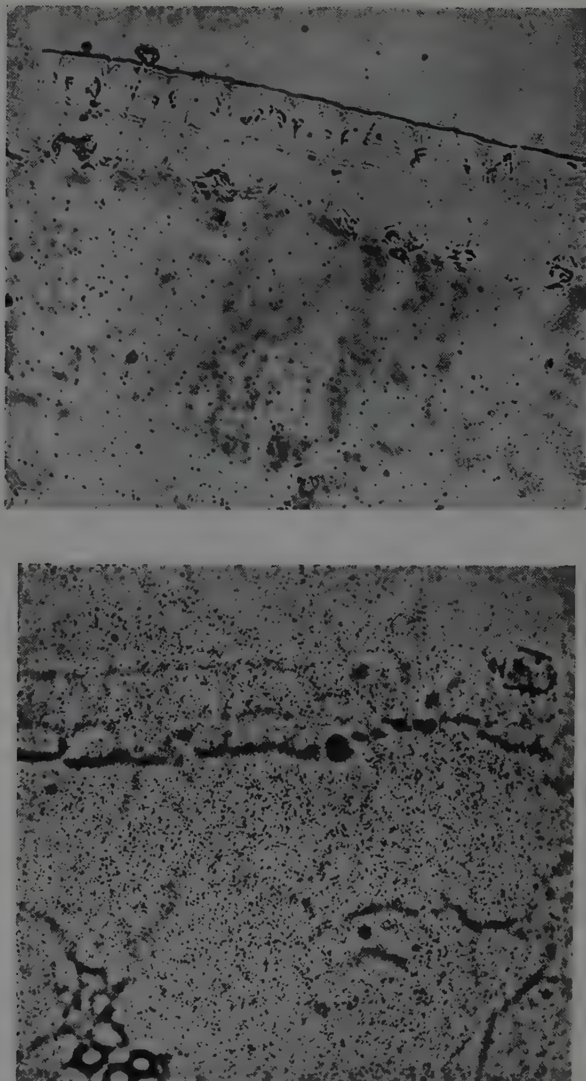

Figure 8

Autoradiographs to show the localization of water-soluble and bound substances which had incorporated C^{14}.

a) After treatment with water, as by normal method.
b) After the apposition technique which retains water-soluble labelled compounds.

drying, allowed investigation of the dried sections and demonstrated striking differences, particularly in the birefringence of the cytoplasm and of unusual cytoplasmic structures.

DISCUSSION

It seems probable from these studies that this new preparatory method preserves plant cells in a life-like condition. It preserves the orientation of the molecules of cell walls and of the chloroplasts, so permitting useful studies to be performed by polarised light microscopy. Unlike the freezing and drying method used so widely by animal cytologists, this technique does not involve treating the tissue with fat solvents so that fatty matter is retained; water-soluble materials can be identified also if a suitable modification of the autoradiographic method is used. Of special interest is the retention, by this procedure, of the very labile mitochondria-like cytoplasmic granules which are characteristic of meristematic cells of roots. This is likely to be of importance because no other reliable method is known by which these organelles can be studied in intact cells, yet such investigations are essential in plant cytochemistry. The significance of these structures arises out of the severe diffusion artifacts which occur when they are destroyed (Chayen 1953, 1959, and in the press) and out of the expectation that they should be sites of great enzymatic activity.

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REFERENCES

Asahina, E., 1956, *Contr. Inst. Low Temp. Sci. Japan*, no. 10, p. 83.

Baker, J. R., 1946, *Cytological Technique*, 2nd Ed., Methuen, London.

Bell, L. G. E., 1952, *Int. Rev. Cytol.*, **1**, 35.

Billingham, R. E., 1954, in *Biological Applications of Freezing and Drying*, Ed. R. J. C. Harris, p. 253, Academic Press, New York.

Chayen, J., 1949, *Nature*, **164**, 930.

Chayen, J., 1952a, *Synip. Soc. Exp. Biol.*, **6**, 290.

Chayen, J., 1952b, *Nature*, **170**, 1070.

Chayen, J., 1953, *Int. Rev. Cytol.*, **2**, 77.

Chayen, J., 1959, *Exp. Cell Res.*, Suppl. 6, p. 115.

Chayen, J., *Exp. Cell Res.*, in the press.

Chayen, J. and Gahan, P. B., 1959, *Quart. J. micros. Sci.*, **100**, 275.

Chayen, J. and Jackson, S. F., 1957, *Symp. Soc. Exp. Biol.*, **10**, 134.

Chayen, J. and Miles, U. J., 1953, *Quart. J. micr. sci.*, **94**, 29.

Chayen, J. and Miles, U. J., 1953, *Stain Technol.*, **29**, 33.

Coons, A. H., Leduc, E. H. and Kaplan, M. H., 1951, *J. exp. Med.*, **93**, 173.

DARLINGTON, C. D., 1937, *Recent Advantages in Cytology*, 2nd ed. Churchill, London.

GUILLERMOND, A., 1941, *The Cytoplasm of the Plant Cell*, translated by L. R. Atkinson, Waltham, Mass. (Chronica Botanica).

HACKETT, D. P., 1955, *Int. Rev. Cytol.*, **4**, 143.

JENSEN, W. A., 1954a, *Stain Technol.*, **29**, 143.

JENSEN, W. A., 1954b, *Exp. Cell Res.*, **7**, 572.

MANCINI, R. E., 1948, *Anat. Rec.*, **101**, 149.

NEWCOMER, E. H., 1940, *Bot. Rev.*, **6**, 85.

SMITH, A. U., 1954, in *Biological Applications of Freezing and Drying*, Ed. R. J. C. Harris, p. 1, Academic Press, New York.

ZIRKLE, C., 1928, *Bot. Gaz.*, **86**, 402.

THE NATURE OF THE TEMPERATURE OPTIMUM AND MINIMUM OF PHOTOSYNTHESIS

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ABSTRACT

According to Egle and Schenk a main reason for the appearance of a temperature optimum for total and net assimilation is seen in the fact, that the respiratory CO_2 is reassimilated at an ever higher partial pressure with increasing temperature, though the CO_2-gradient from the atmosphere to the assimilation organsis diminished. The temperature minimum for net-assimilation lies between —2°C and —7°C or —8°C for evergreen conifers, broad-leaved trees, and some herbaceous plants. It seems that the minimum temperature for net assimilation coincides with the freezing point of their assimilating organs. The minimum temperature for total assimilation coincides with the temperature at which freezing of the needles is complete, at least in *Pinus cembra*.

At present, there is but little detailed knowledge concerning the influence of temperature on the assimilation of CO_2. It is, however, clear that from the purely physiological point of view, a rise in temperature within the range of suitable temperatures brings about an increase in both total and net assimilation*, up to a certain temperature rate, then they decrease again (optimum curve). Selected bibliographies bearing on this point are supplied by Pisek and Winkler (1959), and by Kusumoto (1957). The range of optimum temperatures rises with increasing light intensity and if stomatal regulations do not complicate the picture, the corresponding rates of assimilation increase simultaneously up to a certain light intensity (Müller, 1928; Boysen-Jensen, 1932; Egle and Schenk, 1952, 1953/54; Pisek and Winkler 1959). The optimum temperature range for total assimilation is somewhat higher, more extended, and less sharply defined than in the case of net assimilation. The latter decreases more rapidly with a rise of temperature beyond the optimum, as respiration increases more rapidly than the rate of toytal assimilation.

The experiments of Egle and Schenk (1952, 1953/54) with *Marchantia polymorpha* (in which the size of the breathing pores remains constant) and *Pelargonium zonale* have proved conclusively that the main reason for the appearance of an optimum of total and net assimilation is of a physical nature. With plants assimilating in a closed chamber, under constant conditions of temperature and illumination, the CO_2 content of the air decreased steadily, as the gas was being used up in the process of photosynthesis. However, the concentration of CO_2 in the atmosphere of the chamber was never reduced to zero. The diminution of CO_2 continued only until an

* "Net-assimilation" is sometimes referred to as "apparent assimilation". Total assimilation is the true overall assimilation.

equilibrium was reached at which the CO_2 evolved by respiration was fully reassimilated. With rising temperature the re-assimilation of respiratory CO_2 took place at increasing partial CO_2 pressures. Thus, in open space, any rise in temperature is associated with a diminution of the CO_2 gradient in the surrounding air and, consequently, a reduction in the movement of CO_2 from the surrounding air towards the assimilatory organs, as the low point of the gradient moves up. Increasing light intensity reduces this point.

Data obtained by Alvik (1939) seem to indicate that for seedlings of *Picea excelsa* Link and *Pinus silvestris* Link, the minimum of net assimilation lies a few degrees below 0°C. This is likewise indicated by the findings of Zeller (1951). The investigations of Pisek and Rehner (1958) extended also to samples of branches of other evergreens, such as ericaceous dwarf shrubs and Mediterranean trees and shrubs taken at the onset of spring. The branches were kept for several hours at 0°, −1.5°, −3°, −4°, −5°C, at a light intensity of 3,000 Lux. It was found that the minimum temperature for photosynthesis varies slightly according to species. However, contrary to early exaggerated reports (Jumelle 1892), the minimum temperature for different species ranges between −2° (*Viscum*) and −7° to −8°C (*Olea europaea* and *Laurus nobilis*). The minimum for conifers of northern alpine distribution, such as *Pinus silvestris* and *Picea excelsa*, is about −4°C. At this temperature the majority of branch samples showed some slight absirption of CO_2, but at −5°C evolution of CO_2 predominated over its absorption. This is in agreement with the results obtained by Alvik (1939) and Zeller (1951). Young specimens of *Pinus cembra* from the alpine timber line display a similar reaction to temperature; under the conditions prevailing in their natural habitat, the photosynthetic activity of these trees in autumn is at best sufficient to compensate for the simultaneously occurring respiration as soon as the temperature of the needles drops below −5°C (Tranquillini 1955). Even so, photosynthesis is still possible, as revealed by the fact that evolution of CO_2 (which at temperatures below −°4C replaces CO_2 absorption) is less intense in light than in the dark, when respiration alone takes place. Photosynthesis may be assumed to have stopped altogether only when evolution of CO_2 in light attains the same value as in the dark. The determination of the minimum temperature for total photosynthesis is rather difficult, because of the very low rate of CO_2 release at low temperatures and the difficulties involved in the analysis of the gaseous exchange of plants at such temperatures. However, while repeatedly testing the assimilation of branchlets of *Picea excelsa* and *Pinus cembra* cut from trees growing in the open during the winter months, we observed that following night frosts of −8°C and below, photosynthesis is temporarily suppressed (Pisek and Winter 1958).

In this context, the following results obtained by Tranquillini and Holzer (1958) are of particular interest. The needles of *Pinus cembra* begin to freeze at temperatures between −5°C (March) and −3°C (May) depending on their water content. These months are associated which extremes of low and high hydrature, respectively.

At these temperatures, ice begins to form inside the needles. At $-8°$ all the free water present in the needles (which amounts to about half the total water content of fully saturated needles) freezes. There is no additional ice formation when the temperature is lowered below this level. The minimum temperature for net assimilation coincides at least in the case of *Pinus cembra* with the first freezing of cell fluids in the assimilatory organs, whereas the minimum temperature for total assimilation equals the point at which freezing of the needles is complete. Thus, it is not unlikely that the complete cessation of photosynthesis in the needles is brought about by the freezing, and in particular by the resulting dehydration which involves not only the vacuole but also the living protoplast. There is no doubt that the photosynthetic apparatus is directly affected by these changes. The damage may be produced by slight transitory changes in the structure of the protoplasm, for the inactivation of the photosynthetic apparatus is definitely reversible. Needles of young plants of *Pinus cembra* growing in natural environment, which had passed through a prolonged, low-temperature induced winter dormancy, fully regained the efficiency level of the preceding autumn as spring conditions became firmly established (Tranquillini 1957).

Tests carried out with branches of mature specimens of *Pinus cembra* and *Picea excelsa* showed a similar recovery of assimilatory power (Pisek and Winkler 1958).

The fundamentally reversible character of the inactivation by cold discussed above becomes quite obvious when it is considered that the needles of the observed conifers definitely tolerate ice. Under extreme winter conditions needles of *Picea excelsa* are able to withstand a two-hourly frost at $-36°C$, those of *Pinus cembra* even below $-40°$ without suffering any damage (Ulmer 1937; Pisek and Schiessl 1947). Data concerning the extreme resistance to frost of the other conifers are supplied by Parker (1955), while Mediterranean conifers and other evergreens are discussed by Larcher (1954). It is only when the needles of spruce and *P. cembra* are still young and immature that they freeze to death like the foliage of many deciduous species, whenever ice formation takes place in their tissues even in its initial stages.

Branches cut in the open during the winter at a time when night frosts of -8 to $-10°C$ or below occurred for several successive days, were brought to a constant temperature of 10°C. Under these conditions they required 2-3 days before their photosynthesis surpassed respiration. The photosynthetic apparatus recovered faster when the weather before cutting the samples was less cold. However, the assimilation efficiency never reached its summer rate, not even within a week. Therefore the winter depression of the assimilation efficiency is due not only to the after-effects of frost but also to an inherent state of dormancy due to climatic conditions.

Freezing temperatures in the region of the minimum for net assimilation reduce the ability of plants to assimilate CO_2 for a period exceeding the duration of the frost. Thus, whenever frost occurs — as it generally does — in the course of the night, the rate of photosynthesis on the following warm day remains below the value attained under otherwise similar conditions prior to the frost. Yet, the effect of such frost

is far less pronounced and lasts considerably less than the effect of lower temperatures (Tranquillini 1957). The minimum value reached by the net assimilation rate may well be determined by the fact that already the inception of ice formation in the needle may directly, though only partially, inactivate the photosynthetic apparatus of the needle. Alternatively, inability of the needle to pass the point of compensation at which photosynthesis equals respiration may be due to the fact that such frosts bring about a complete closure of the stomata, which prevents the intake of atmospheric CO_2 by the plant. Or else, both these factors may act simultaneously. Further experiments are required to decide this point. Such experiments would also be expected to broaden the factual basis and to confirm the theory concerning the nature of the temperature minimum of photosynthesis proposed in this paper.

In conclusion, it should be mentioned that preliminary tests with leaves of *Olea* and *Laurus* yielded comparatively low freezing points of $-9°$ to $-10°C$ (Larcher, unpublished). It will be recalled that the minimum temperatures for net assimilation in *Olea* and *Laurus* (-7 to $-8°C$) are likewise surprisingly low. The values obtained for the freezing point in these experiments nearly coincide with the temperature at which the foliage of these plants suffers in winter irreversible damage. These Mediterranean evergreens cannot tolerate ice formation in the tissues under any circumstances. Thus it appears that in these plants, too, the minimum temperature for net assimilation coincides with the freezing point of their assimilating organs. It would appear that not only in the case of *Pinus cembra* and other conifers, but more generally, the minimum temperature for net assimilation is linked with the freezing point of the assimilating organs.

REFERENCES

ALVIK, G., 1939, Über die Assimilation and Atmung einiger Holzgewächse im westnorwegischen Winter, *Meddelelse 22 fra Vestlandets forstlige Forsoksstation.*

BOYSEN-JENSEN, P., 1932, *Die Stoffproduktion der Pflanzen*, G. Fischer, Jena.

EGLE, K. and SCHENK, W., 1952, Untersuchungen über die Reassimilation der Atmungskohlensäure bei der Photosynthese, *Beitr. Biol. Pfl.*, **29**, 75.

EGLE, K. and SCHENK, W., 1953/54, Der Einfluss der Temperatur auf die Lage des CO_2-Kompensationspunktes, *Planta* **43**, 83.

JUMELLE, H., 1892, *Rev. Gen Bot.*, **4**, 168. (quoted by Zeller, 1951).

KUSUMOTO, T., 1957, Physiological and ecological studies on the plant production in plant communities. 3. Ecological consideration of the temperature-photosynthesis-curves of evergreen broadleaved trees, *Japan. J. Ecology*, **7**, 126.

LARCHER, W., 1954, Kälteresistenz mediterraner Immergrüner und ihre Beeinflussbarkeit, *Planta*, **44**, 607.

MUELLER, D., 1928, Die Kohlensäure-Assimilation der arktischen Pflanzen und die Abhängigkeit der Assimilation von der Temperatur, *Planta* **6**, 22.

PARKER, J., 1955, Annual trends in cold hardiness of ponderosa pine and grand fir, *Ecology*, **36**, 377.

PISEK, A. and REHNER, G., 1958, Temperaturminima der Netto-Assimilation von mediterranen und nordisch-alpinen Immergrünen. *Ber. Deutsch. Bot. Ges.*, **71**, 88.

PISEK, A. and SCHIESSL, R., 1947, Die Temperaturbeeinflussbarkeit der Frosthärte von Nadelhölzern und Zwergsträuchern an der alpinen Waldgrenze, *Ber. Naturw.-Mediz. Ver. Innsbruck*, **47**, 33.

PISEK, A. and WINKLER, E., 1958, Assimilationsvermögen und Respiration der Fichte (*Picea excelsa* Link) in verschiedener Höhenlage und der Zirbe (*Pinus Cembra* L.) an der alpinen Waldgrenze, *Planta*, **51**, 518.

PISEK, A. and WINKLER, E., 1959, Licht und Temperaturabhängigkeit der CO_2–Assimilation von Fichte (*Picea excelsa* Link), Zirbe (*Pinus Cembra* L.) und Sonnenblume (*Helianthus annuus* L.), *Planta*, **53**, 532.

TRANQUILLINI, W., 1955, Die Bedeutung des Lichts und der Temperatur für die Kohlensäure-Assimilation von *Pinus Cembra* Jungwuchs an einem hochalpinen Standort, *Planta* **46**, 154.

TRANQUILLINI, W., 1957, Standortsklima, Wasserbilanz und CO_2–Gaswechsel junger Zirben (*Pinus Cembra* L.) an der alpinen Waldgrenze, *Planta*, **49**, 662.

TRANQUILLINI, W. and HOLZER, K., 1959, Über das Gefrieren und Auftauen von Koniferennadeln, *Ber. Deutsch. Bot. Ges.*, **71**, 143.

ULMER, W., 1937, Über den Jahresgang der Frosthärte einiger immergrüner Arten der alpinen Stufe, sowie der Zirbe und Fichte, *Jb. wiss. Bot.*, **84**, 553.

ZELLER, O., 1951, Über Assimilation und Atmung der Pflanzen im Winter bei tiefen Temperaturen, *Planta*, **39**, 500.

CORRIGENDA

Section D, Botany. Volume 7. Number 3–4

p. 222, l.	1 from top	instead of	Aphylophorales	read Aphyllophorales
p. 222 l.	2 from top	instead of	7 to Gasteromycetes	read 6 to Gasteromycetes
p. 227, l.	17 from top	instead of	*Russula alutaceae*	read *Russula alutacea*

p. 235, before line 5 from top add the heading GASTEROMYCETES

p. 242, figure E is upside down

p. 243, l. 2 from bottom instead of *Russula alutaceae* read *Russula alutacea*

p. 245, instead of Plate IV read Plate VI; the legend belonging to this plate has been printed on p. 247

p. 247, instead of Plate VI read Plate IV; the legend belonging to this plate has been printed on p. 247

• Volume 8D, Number 3–4, April 1960

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section D
BOTANY

Bull. Res. Counc. of Israel. D. Bot.

Continuing the activities of the
Palestine Journal of Botany,
Jerusalem and Rehovot Series

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TO

VOLUME 8D

The Weizmann Science Press of Israel • Jerusalem • ירושלים • מוסד ויצמן לפרסומים במדעי הטבע ובטכנולוגיה בישראל

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3. TAYLOR, G. I., 1932, *Proc. roy. Soc.*, A138, 41.

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